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ORIGINAL ARTICLES

THE TOXEMIA OF SEVERE SUPERFICIAL BURNS*

BY BRUCE ROBERTSON, B.A., M.B., (TOR.) AND GLADYS L. BOYD, M.B. (TOR.), TORONTO, CANADA

SHOCK, hemorrhage and toxemia have each been offered as theories to explain the cause of death in superficial burns. As early as 1868, Wertheim[1] believed death to be due to a toxin circulating in the blood, and six years later Ponfick[2] came to the same conclusion. Shortly after, a toxic substance was found in the urine of burned animals by various investigators; Lustgarten[3] thought this toxin was muscarine, or a similar substance. Reiss[4] considered it a pyridine base. Ajello and Parascandello[5] found a toxic substance both in the blood and urine of burned patients; they regarded the poison as a ptomaine. During the ten years ending in 1914, Herman Peiffer[6, 7] of the University of Gratz, carried on extensive research into the etiology of death in burns. He came to the conclusion that there was a poison produced at the site of the burn which circulated in the blood. The chemical nature of the toxin he considered unknown, but thought it of a nucleoprotein nature, somewhat resembling snake venom. He not only noted the presence of a toxic substance in the urine of burned animals, but found an increased nonspecific proteolytic ferment in it. This latter finding was also reported by Ferrae,[8] but Parascandello was unable to corroborate their findings.

(1) BURNED TISSUE.—A. *Skin Burns.*—Young anesthetized rabbits were used in these experiments. Older anesthetized rabbits and dogs were tried, but difficulty in producing a superficial skin burn on the thick skin, rendered them of little use for our purpose. An area of skin over the side of the thorax

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and abdomen, about 40 by 70 square centimeters in size, was prepared by shaving and the use of barium sulphite as a depilatory. The animal was in all cases fully anesthetized with ether, and a burn produced by the application of hot metal plates to the prepared area. The technic used by Eijkman and van Hoogenhyze[9] of alternate dipping of the ears of the anesthetized animal into hot and cold water was used in three cases, but the animals all died within a few hours of primary shock. A similar cause of death in their animals is indicated by their protocols.

Typical symptoms followed the production of the burn in all cases. Immediately following the operation under ether anesthesia, there is a period of

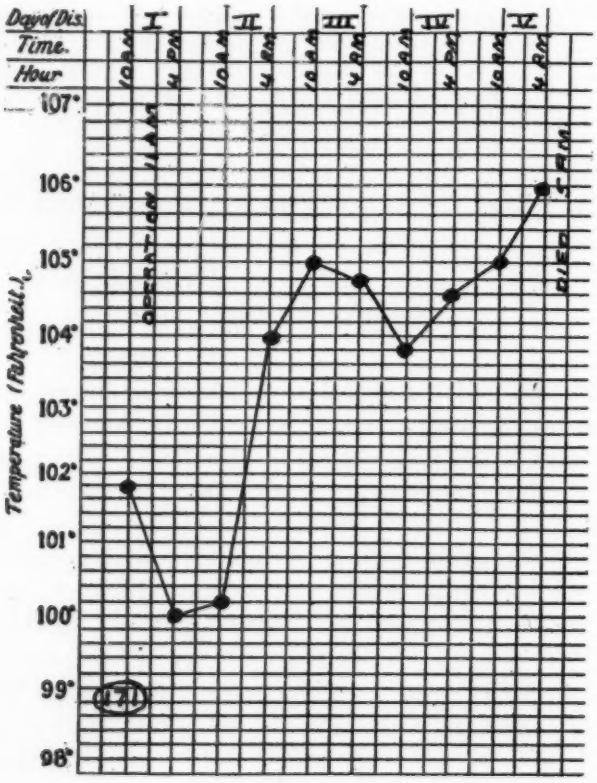

Fig. 1.

primary shock, evidenced by a circulatory collapse and a fall in temperature. This phase lasted six to eight hours. During the next ten to twenty-four hours the animals did not appear very ill. In twenty-four to thirty-six hours after the burn toxic symptoms appeared. The temperature rose to 104°-106° F. (Normal temperature of a rabbit 100°-102° F.) Food was refused. The animal was drowsy or excitable, or exhibited alternate periods of each. Convulsive twitchings, and in a few cases general convulsions, were noted. Some animals had diarrhea with mucus in their stools. Two had melena. Hemoglobinuria, lasting two or three days, was present in three cases. The total nonprotein nitrogen of the blood was increased 40 to 50 per cent over the

normal amount; this was chiefly due to the relatively greater increase of urea nitrogen to 80 or 90 per cent of the total nonprotein nitrogen. These symptoms and signs, in the nonfatal cases, persisted two to three weeks, with gradual improvement after the first week. Eight cases proved fatal; in two cases death occurred in the first eight hours and was attributed to primary shock; in five cases it occurred in from thirty-six hours to five days after the operation and was due to toxic shock; one case died three weeks after the burn from secondary infection.

B. *Subcutaneous Tissue Burns.*—In five anesthetized rabbits, flaps of skin from the side of the thorax and abdomen were dissected up, and the sub-

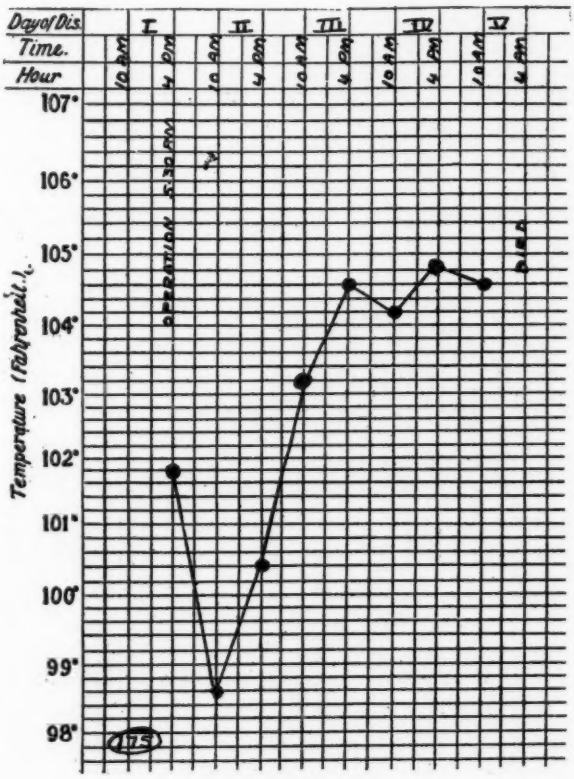

Fig. 2.

cutaneous tissue burned with the hot metal plates, the flap of skin was then sutured in place. Results were obtained identical with those produced by burning the skin.

C. *Muscle Burns.*—Two methods of producing muscle burns were used with different results. Injection of 20 cubic centimeters of water of 100° C. into the thigh muscles of the anesthetized rabbits was done in two cases and produced no symptoms, but those of a slight degree of primary shock ensued. Eijkman and van Hoogenhyse[9] used this method and failed to produce any evidence of burn toxemia. From their experiments they concluded that the toxic substance was not produced in muscle burns. Secondly, we exposed

TABLE I

RABBITS—BURNS

NO.	DATE	WEIGHT	METHOD	AREA	SYMPTOMS	RESULT	AUTOPSY	N.P.N.
170	4/16/21	1490	Plates	5 x 5½	Primary shock. Drowsy and refused food about 3 days. Quiet for another week. Temp. 103-104 (102 before op.)	Clinical Recovery	Bled to death 4/28. Fatty degen. liver. Congestion of bowel wall.	
171	4/16/21	1590	Plates	4 x 5½	Primary shock. Refused food and drowsy. Wt. 4/20 1340 gms. Temp. 105-106. Still very ill. Bled to death 4/20/21.	Death	Hem. exudate in perit. cavity. Cloudy swelling liver and kidneys. Spleen looks normal. Congest. and petech. hem. in bowel—small especially.	
172	4/16/21	1585	Plates	4 x 5.5	Primary shock. Appeared fairly well during evening. Next a.m. drowsy and refusing food. Wt. 1380 on 4/20. Temp. 104-105 (101 before op.) Bled to death 4/20/21.	Death	Same as 171 except for absence of hem. exudate in peritoneum.	
L. B.	4/16/21	1310	Scalding ears in water at 65° C. 3 mts. then 15°, 3 mts. Repeated 3 times.		Marked primary shock. Ears swollen twice size in 10 minutes. Quiet and muscular twitchings for 4 days following. Temp. 103-104, (102).	Recovery		
773	4/18/21	3550	Plates	5 x 5.5 Subcutaneous tissue	Primary shock. Drowsy and frequent muscular twitchings for 4 days. Temp. 103-105.8 (102). Died 4 days after.	Death	Cloudy swelling liver and kidney. Suprarenals large?—hemorrhagic. Petechial hemorrhages in bowel wall.	
174	4/18/21	2480	Plates	8 x 5.5 Subcutaneous tissue	Primary shock. Very quiet and drowsy for 1 week. Ate very little. Temp. 103-105.	Recovery	Killed 4/28 because of secondary infection of wound.	
175	4/18/21	2230	Plates	7 x 5.5 Subcutaneous tissue	Primary shock. Very ill after. Water diarrhea with mucus. Eats very little. Temp. 103-104 (103). Died night of 20th.	Death	Congestion mes. vessels. Petech. hem. and ulceration in bowel. Perforation of ileum. Cloudy swelling of liver and kidneys.	

TABLE I—CONT'D.

RABBITS—BURNS

NO.	DATE	WEIGHT	METHOD	AREA	SYMPTOMS	RESULT	AUTOPSY	N.P.N.
Black	2/7/21	3570	Scalding 1½ m.	15 x 7	None. Dry gangrene of area	Recovery		30
Black	2/14/21		metal plate	4 x 5½	None. Dry gangrene of area			
Brown	2/6/21	1435	Scalding	7 x 10	Insufficiently burned	Recovery		36.9
White	2/7/21	1340	Scalding 1.min.	11.5 x 19.5	Insufficiently burned			
Brown	2/7/21		Scalding	Thigh muscle	Insufficiently burned			
G. & W.	2/14/21	1700	Plates	6 x5.5	Dry gangrene	Recovery		25.4
White	2/14/21	1750	Plates	5 x 5.5	Dry gangrene			
B. & W.	4/4/21	1370	Plates	5.5 x 3 under surface of skin	Primary shock. Appeared quiet and ate very little for 10 days after. Temp. constantly above 103 for 2 weeks when it became lower and rabbit appeared better. Passed small amount blood in stool twice.	Recovery		43.2
G. & W.	4/4/21	1260	Plates	5½ x 4 cm. subcutaneous tissue	Primary shock well marked. Had one convulsion about 6 hours after. Died about 36 hours later. Temp. 106.	Death	No gross changes in organs. Petechial hemorrhages in bowel wall. Mesenteric vessels engorged. Fibrinous plugs in pulmonary vessels. Necrotic areas around portal vein.	44.0
159	4/7/21	1220	Plates	4 x 5½ skin thin and fine	Primary shock. Drowsy and refused all food 2 days. Ill about 2 weeks. Temp. 103 during this time. Blood in stools once.	Clinical Recovery	Bled to death 4/28. No gross changes observed other than congestion. Microscop. some necrosis in bowel wall. Degenerative changes in parenchyma of organs.	
160	4/7/21	1330	Plates	5 x 5.5	Primary shock. Refused food 24 hrs. Quiet and drowsy 2 days. Quiet for one week. Temp. 103-103.4.	Recovery		
161	4/7/21	1230	Plates	Thigh muscle	Primary shock. Very drowsy and refused food 48 hrs. Apparently quite ill and ate very little for over a week. Temp. 103-104.4.	Recovery		
168	4/14/21	4270	Injection boiling water. 20 c.c.	Thigh muscle	Primary shock. Appeared slightly less active for a couple of days. Temp. 102-103 (103 before op.)	Recovery		
169	4/15/21	2230	"	Thigh muscle	"	Recovery		

All operations on animals in these experiments were done with the animal under deep ether anesthesia.

the thigh muscles of a rabbit, fully anesthetized, and burned them by the application of the hot metal plates, and then closed the wound up. Four animals treated in this manner exhibited all the typical symptoms of burn toxemia, but all ultimately recovered.

D. *Transplantation of Burned Skin.*—In twelve rabbits, fully anesthetized, the burned skin was removed at different intervals after the burning and transplanted to healthy rabbits, the skin from the latter being used to cover the wound in the burned animals. Secondary infection occurred in only one case, the transplants taking in all other cases. Removal of the burned skin, less than eight hours after the animal was burned, saved the animal from all toxic symptoms, but removal subsequent to this time failed to prevent the development of toxemia in the animal. The rabbits receiving the transplants of burned skin exhibited toxic symptoms in all cases within an hour of the operation. Similar results were obtained by Vogt,[10] who used parabiosis, followed by burning of one of the animals, and their separation at different intervals after the operation. He noted toxic symptoms in both animals, unless the separation was done within twelve hours after the burning.

When removing the burned skin, a certain amount of edema and congestion of the subcutaneous tissue was seen within one hour after the burning. After the first two or three hours a bloodstained gelatinous material was present between the skin and subcutaneous tissue. This substance increased in amount up to twenty hours after, and then gradually disappeared.

PATHOLOGY

General hyperemia of the abdominal organs was noted in all those cases dying within a week of the production of the burn. In two cases, the peritoneal cavity contained a hemorrhagic transudate. Petechial hemorrhages in the bowel mucosa were common, particularly in that portion of the ileum just above the cecum. Changes in the abdominal organs of an acute parenchymatous nature were seen microscopically. Ecchymosis in all cases, and in some ulceration of the mucosa of the bowel was seen. Death in one case was caused by a perforation of the lower end of the ileum. The lungs were congested, and some of the pulmonary vessels contained fibrinous plugs, some of which showed partial organization. The heart muscle appeared normal when death occurred early, but was the seat of fatty degeneration when the animal lived a week or more. Changes in the glandular organs, viz., swelling and focal necrosis, described by Bardeen[11, 12] and later Weiskotten[13] as typical of burn toxemia, were seen only twice in our cases. The only abnormality noted in these organs in most cases was a varying amount of interstitial hemorrhage and degenerative changes in the parenchyma.

INJECTIONS OF TOXIN

1. Blood.—A. *Serum.*—The injection of blood serum obtained from children suffering from burn toxemia into guinea pigs and rabbits caused no symptoms until such large doses were given that an anaphylactic reaction occurred. Control animals given normal serum died just as frequently and exhibited the same symptoms as those receiving the burn serum.

B. *Whole Blood.*—Citrated whole blood was next injected intraperitoneally into rabbits and pigs. No anaphylactic phenomena followed the injection of normal whole blood in doses up to 15 cubic centimeters in 300 gram pigs. Doses of 2 or 3 cubic centimeters of whole blood from rabbits

TABLE II

BURNS—DOGS

NO.	DATE	WEIGHT	METHOD	AREA	RESULT
Small bl. & wh.	4/11/21	4450	Plates skin	5½ x 5½	Dog never appeared ill and showed no elevation of temperature. Skin began to peel off 4 days after burn.
Collie	4/12/21		Plates skin	5½ x 20	No symptoms at all. Skin showed no burn changes. Temperature normal.
Br. & Bl.	4/12/21		Plates skin	thigh muscle	No primary shock. Dog quieter than usual for a few days. Temperature 100-102 for 3 days (100).
Bl. & Wh. small	4/19/21		Plates skin	10 x 5.5 sub. tissue	Primary shock. Dog appeared only a little less quiet than usual. No fever.
Bk. & Wh large	4/19/21		Plates skin	14 x 5.5 sub. tissue	Primary shock well marked for 6 hours, after which dog appeared O. K.
			BURNS—RABBITS		
199	5/4/21	1385	Plates skin	7 x 5.5	Primary shock well marked—10 hours. Rabbit never appeared more than slightly ill, following recovery from shock. Fever 103-103.5 for 9 days.
200	5/4/21		Plates skin	6 x 5.5	Died of primary shock shortly, 15 minutes after burning.
201	5/4/21		Plates skin	6 x 5.5	Died of primary shock shortly, 15 minutes after burning.

TABLE III

TRANSPLANTS—BURNED SKIN—RABBITS

NO.	WT. GM.	BURN OR TRANSPLANT	SKIN REMOVED	RESULT	AUTOPSY
403	1470	Burned	21 hours after	Animal toxic for 10 days. Ultimate recovery.	
404	1460	Transplant 4¼ hrs. after burning from rabbit 401		Toxic for 2 weeks.	Death from secondary infection 3 weeks later.
401	1240	Burned	4¼ hours after	Primary shock, slight toxemia 2 days, complete recovery.	
402	1130	Burned	6½ hours after	Slight toxemia for 2 days; recovery.	
405	1350	Transplant from 402		Toxemia with blood diarrhea immediately; death on 5th day.	Liver—fatty degeneration—necrotic areas around portal vein. Kidneys—cloudy swelling. Lungs and bowel congested.
406	2270	Transplant from 403.		No primary shock. Toxic symptoms almost at once. Hemoglobinuria for 2 days. Recovery.	

or children suffering from burn toxemia injected into pigs resulted in the onset of toxic symptoms consisting of alternate periods of drowsiness and excitability, weakness of the hind legs, convulsions, and in five cases, death. Autopsy findings in the fatal cases were identical with those seen in burned animals.

C. *Dialysed Blood.*—Normal blood and that obtained from cases of burn

TABLE IV

BLOOD INJECTIONS—PIGS

NO.	WT.	SUBSTANCE	METHOD	SYMPTOMS	RESULT	AUTOPSY
182	340	2.5 c.c. whole blood (burn case)	Intraper.	Convulsions or drowsiness 10 hours. Weakness hind legs and refused food 3 days.	Recovery	
183	285	5 c.c. whole blood (burn case)	Intraper.	Convulsions 10 hours. Drowsy and refusing food 2 days.	Recovery	
184	300	5 c.c. whole blood (normal)	Intraper.	None	Recovery	
185	285	5 c.c. whole blood (normal)	Intraper.	None	Recovery	
196	165	2.5 c.c. whole blood (burn case)	Intraper.	Drowsiness 5 hours followed by convulsions for 5 or 6 hours. Apparent paralysis of hind legs 1 week, died in 1 week.	Death	Congestion of all abdominal contents. Petechial hemorrhages in bowel. Some fatty degeneration of liver and spleen.
413	190	3 c.c. whole blood (burn case)	Intraper.	Much like 196	Death	Congestion of all abdominal contents. Petechial hemorrhages in bowel. Some fatty degeneration of liver and spleen.
198	580	4.5 c.c. whole blood (burn case)	Intraper.	Muscle twitchings and irritability at once. In 5 hrs. drowsiness and weakness hind legs. Refusal of all food 3 days. Gradual improvement.	Recovery	
200	310	10 c.c. whole blood (normal)	Intraper.	None	Recovery	
151	330	10 c.c. diffusate (burn blood)	Intraper.	None	Recovery	
152	330	10 c.c. supernatant fluid after centrifuging residue (burn)	Intraper.	None	Recovery	
153	280	10 c.c. normal supernatant fluid.	Intraper.	None	Recovery	
	265	10 c.c. saline sol. of shaken corpuscles from burn blood	Intraper.	General convulsions in 1 minute, lasted 8 hours. Pig appeared comatose; gradual improvement.	Recovery	

TABLE IV—CONT'D.
BLOOD INJECTIONS—PIGS

NO.	WT.	SUBSTANCE	METHOD	SYMPTOMS	RESULT	AUTOPSY
154	300	10 c.c. residue from normal blood.	Intraper.	None	Recovery	
155	295	5 c.c. burn residue	Intraper.	Alternate excitability and drowsiness for 24 hours	Recovery	
	290	5 c.c. burn residue	Intraper.	Convulsions and drowsiness alternating 2 days.	Death	Congestion of contents of abd. cavity. Parenchymatous degenerative changes in solid organs. Petechial hemorrhages in bowel.
	295	5 c.c. burn residue	Intraper.	Convulsions or drowsiness 3 days.	Death	Congestion of contents of abd. cavity. Parenchymatous degenerative changes in solid organs. Petechial hemorrhages in bowel.
132		5 c.c. serum from burn case.	Intraperitoneal	None	Recovery	
133		2.5 c.c. serum from burn case.	Intraperitoneal	None	Recovery	
131		5 c.c. normal human serum	Intraperitoneal	None	Recovery	
126		10 c.c. burn serum.	Intraperitoneal	Slight drowsiness 12 hrs.	Recovery	
127		10 c.c. normal serum	Intraperitoneal	None	Recovery	
129		20 c.c. burn serum	Intraperitoneal	General convulsions with respiratory distress off and on for 3 days.	Death	
		20 c.c. normal serum	Intraperitoneal	Same as 129	Death	
		30 c.c. burn serum	Intraperitoneal	Convulsions — death in 1 hour.	Death	
		30 c.c. normal serum	Intraperitoneal	Convulsions — death in 3 hours.	Death	
		15 c.c. burn serum	Intraperitoneal	Convulsions in ½ hour. Death in 7.	Death	
	240	10 c.c. diffusate (burn blood)	Intracardial.	Slight twitchings ½ hour.	Recovery	
	310	10 c.c. diffusate (normal blood)	Intracardial.	None	Recovery	
	270	5 c.c. diffusate (burn blood)	Intracardial.	Twitching for a few minutes.	Recovery	

toxemia were dialysed against distilled water, through heavy parchment paper, and both the residue and diffusate used for animal inoculation.

(1) Diffusate.—Intracardial injections of 10 cubic centimeters of the diffusate from toxic cases into 250 to 270 gram guinea pigs proved absolutely innocuous. Intraperitoneal injections of larger doses also failed to cause any symptoms.

(2) Residue.—The portion of the blood left in the parchment paper was centrifuged and the supernatant fluid separated from the sediment by

decantation. Injection into the peritoneal cavity of 10 cubic centimeter doses of the supernatant fluid into two pigs of 280 and 310 grams produced no symptoms. The sediment was shaken for three hours with equal volumes of Lloyd's reagent and normal saline. The fluid was then squeezed out by pressure of the mixture in a canvas bag. This fluid was put through a Berkfeld filter and used for inoculation. Intraperitoneal injection of doses up to 20 cubic centimeters into 300 gram pigs of the normal blood proved innocuous, but 5 cubic centimeter doses of the blood from the burn cases proved toxic in five pigs of about 300 grams, and fatal in one. The symptoms of toxemia were the same as in the animals with burned tissue.

2. Skin Extracts.—The skin extracts were prepared as follows: Pieces of burned skin, 280 to 300 square centimeters in size, were used. The skin was freed from subcutaneous tissue and fat and washed free from blood. It was then finely ground with sea sand in a meat chopper. This was well covered with freshly distilled water or normal saline, and allowed to stand twelve hours. It was placed in a canvas bag and the fluid squeezed out in a press. Four volumes of absolute alcohol were added to the fluid obtained. This was filtered. The extract was concentrated, and the alcohol removed by evaporation in a vacuum, at a temperature not exceeding 40° C. A clear yellowish, slightly viscid fluid was thus obtained. This was filtered through a Berkfeld filter, and then used for inoculation. Extracts of normal skin prepared in the same way were used for controls.

Ten cubic centimeter doses of extract of normal skin were injected intraperitoneally into four pigs of 250, 280, 260 and 263 grams, respectively. No ill effects whatever followed these injections. Similar negative results were obtained following the injection of extracts of normal skin burned postmortem.

The injection of extracts of burned skin from animals or children resulted in the development of the symptom complex of burn toxemia. Doses of 1 to 2 cubic centimeters, representing roughly $\frac{1}{60}$ to $\frac{1}{50}$ of the total extract obtained from pieces of skin 280 to 300 square centimeters in size, were sufficient to cause these symptoms in twenty pigs of 275 to 300 grams in weight. Eight of the twenty died in general convulsions. Necropsy findings were the same as those seen in superficial burns.

Boiling the extract for two minutes before injecting altered the effect produced. Nervous symptoms of the same character ensued but the animals (seven in number) all recovered. If the animals were killed within two or three days of the injection, while still exhibiting neurotoxic phenomena, little evidence of any change in the tissues could be found.

Portions of two of the extracts were dialysed against distilled water through heavy parchment paper. Doses of the diffusate comparable to 2 to 3 cubic centimeters of the whole extract were injected intraperitoneally into five pigs of 280 to 300 grams. Definite neurotoxic symptoms ensued but as in the case of boiled extract, little evidence of necrotoxic changes in the organs could be found.

Alcoholic extracts proved a little less toxic than did saline ones. Keep-

ing the extract in the ice chest for three weeks did not cause any apparent diminution in its toxicity.

3. Liver Extracts.—Liver extracts from burned and normal animals were prepared in the same manner as the skin extracts. Those from burned animals produced much the same symptoms as did those from normal animals when injected into pigs. In both cases the extracts were toxic.

4. Spinal Fluid.—Fourteen cubic centimeters of spinal fluid from a child suffering from a severe burn toxemia were injected intraperitoneally into a pig. Convulsive twitchings and a few general convulsions followed almost immediately, but lasted only a few hours. In twelve hours the pig appeared perfectly normal.

5. Contents of Burn Blisters.—The contents of burn blisters were obtained by means of a sterile syringe before the application of any medication to the skin. Intraperitoneal injection into five pigs, all less than 250 grams in weight, of doses of 1, 3 and 5 cubic centimeters failed to produce any definite symptoms. The pigs appeared a little restless for a few hours, and then behaved as normal animals would.

ANTIBODY PRODUCTION

Attempts to stimulate the production of antibodies were made by injecting sublethal doses of skin extract or toxic blood, and following such doses by the administration of gradually increasing doses of the toxic substance as soon as symptoms produced by the first injection had disappeared. Such experiments in eight animals failed to indicate the production of any protective substance by the animal. The dosage could be increased to some extent, but administration of the dose found to be fatal in other animals, resulted in death in the treated animals also. Kutscher and Heyde[14] found that small burned areas sensitized the animal to injections of toxin.

NATURE OF THE TOXIC SUBSTANCE

The diversity of opinions concerning the chemical nature of the toxin produced in burns has already been indicated. In brief, it has been regarded as a ptomaine, nucleoprotein, methyl guanidine, muscarine, a pyridine base. Its proteolytic nature has been indicated by the finding of an increase in the proteolytic activity of the serum and urine of burn cases by Pfeiffer[7] and Ferrae.[8] The increase in the nonprotein nitrogen of the blood, particularly of the urea nitrogen which we noted above, is another evidence of increased protein catabolism.

The preparation of our skin extracts showed that the toxin was soluble in water or saline, not precipitated by alcohol, slightly soluble in alcohol, nonvolatile, not destroyed by temperature of 40° C. or less, and able to pass through a bacteria tight filter.

Boiling the extract, while altering the effect produced, did not wholly destroy the toxin, as nervous symptoms equal in kind and severity with those produced by unboiled extract followed. A similar effect was obtained when the extracts were dialysed, the diffusate in that case producing the same effect when injected as did the boiled extract. These experiments lead us

to conclude that the toxin consisted of two portions, much like snake venom, one part of which was thermostabile and diffusable, and exerted its toxic action on the nervous system, while a second portion was thermolabile and nondiffusable and necrotoxic in its effects.

Experiments on the skin extracts and the fluid obtained from the sedi-

TABLE V

SKIN EXTRACTS—PIGS

NO.	WT.	SUBSTANCE	METHOD	SYMPTOMS	RESULT	AUTOPSY
157	265	10 c.c. saline extract normal skin.	Intraper.	A little quiet for 2 hrs.	Recovery	
	270	8 c.c. saline extract normal skin.	Intraper.	None	Recovery	
	290	10 c.c. saline extract normal skin.	Intraper.	None	Recovery	
158	260	8 c.c. saline extract normal skin burned p. m.	Intraper.	None	Recovery	
	265	10 c.c. saline extract normal skin burned p. m.	Intraper.	None	Recovery	
181	315	5 c.c. saline extract burned skin heated to 100° C. 2 minutes.	Intraper.	Drowsy and irritable, refused food.	Recovery	
184	205	5 c.c. saline extract burned skin.	Intraper.	Convulsive twitchings almost immediately. Died during the first 24 hours.	Death	Hemorrhagic transudate in abdomen. Mesenteric vessels congested. Petechial hemorrhages in stomach wall. Suprarenals hemorrhagic. Kidneys and liver cloudy swelling.
422	390	5 c.c. skin extract (skin removed from animal immediately after burning).	Intraper.	Collapse and convulsions lasting 1 hour. Rapid recovery.	Recovery	
423	390	5 c.c. skin extract (skin removed from animal 3 hrs. after burning)	Intraper.	Convulsions and collapse immediately. Died in 3 hrs.	Death	Congestion of abdominal contents. Petechial hemorrhages in bowel. Fibrinous plugs in pulmonary vessels, degenerative changes in solid organs.
426	285	2 c.c. skin extract (same as 423 had).	Intraper.	Convulsions and collapse at once lasting 12 hrs. Drowsiness, irritability and refusal of food 6 days. Died on 7th day.	Death	Congestion of abdominal contents. Petechial hemorrhages in bowel. Fibrinous plugs in pulmonary vessels, degenerative changes in solid organs.

TABLE V—CONT'D.

SKIN EXTRACTS—PIGS

NO.	WT.	SUBSTANCE	METHOD	SYMPTOMS	RESULT	AUTOPSY
427	290	2 c.c. skin extract (skin removed from animal 11 hrs. after burning).	Intraper.	Collapse and general convulsions at once. Death in 10 hrs.	Death	Congestion of abdominal contents. Petechial hemorrhages in bowel. Fibrinous plugs in pulmonary vessels, degenerative changes in solid organs.
429	275	2 c.c. skin extract (skin removed from animal 18 hrs. after burning).	Intraper.	Convulsive twitchings, irritability and drowsiness for 6 days. Death on 7th.	Death	Congestion of abdominal contents. Petechial hemorrhages in bowel. Fibrinous plugs in pulmonary vessels, degenerative changes in solid organs.
433	280	3 c.c. saline extract burned skin, boiled 2 minutes.	Intraper.	Excitability and some drowsiness for 3 hrs.	Recovery	Killed in 1 week. Nothing abnormal found. No evidence of reaction at site of injection.
408	200	7 c.c. saline extract burned skin, boiled 2 minutes.	Intraper.	Excitable and later drowsy for a couple of days.	Recovery	Killed on 5th day. Findings same as in 433.
407	195	3 c.c. saline extract burned skin, boiled 2 minutes.	Intraper.	Excitable and later drowsy for a couple of days.	Recovery	Killed on 5th day. Findings same as in 433.
467	210	10 c.c. diffusate from burned skin extract.	Intraper.	Convulsions and collapse starting 15 minutes after injection and lasting 5 hrs. Drowsiness and irritability 2 days.	Recovery	Killed on 4th day. Necropsy findings negative.
493	205	10 c.c. diffusate from burned skin extract.	Intraper.	Convulsions 6 hrs. Drowsy, irritable refusing food 72 hrs. Gradual recovery.	Recovery	Same as 467.

TABLE VI

CONTENTS OF BURN BLISTERS

NO.	WEIGHT	SUBSTANCE	METHOD	SYMPTOMS	RESULT	AUTOPSY
437	570	3 c.c. contents burn blister	Intraperitoneally	None None	Recovery Recovery	
438	520	3 c.c. contents	Intraperitoneally	None	Recovery	
414	190	2 c.c. contents	Intraperitoneally	None	Recovery	
415	175	1.5 c.c. contents	Intraperitoneally	None	Recovery	
416	160	6 c.c. contents	Intraperitoneally	None	Recovery	

ment of blood from burn cases were made to determine the nature of the protein-split products present. The nondiffusability of a portion of the toxin indicated its colloidal nature. The toxin was not coagulated by heat, nitric or picric acid. When the nitric acid was added drop by drop a white pre-

cipitate formed which disappeared on heating. The biuret test gave a purple ring. Sulphur was tested for with lead acetate and sodium hydroxide, but the tests were negative. Salting out of the solutions of toxin showed them to contain primary and secondary proteoses. The diffusate contained only the latter.

CONCLUSIONS

1. Death from burning occurring within the first twenty-four hours is practically always due to primary shock; later than this, it is due to toxic shock.

2. There is a toxin produced in burned tissue, in larger quantities in skin burns, which circulates in the blood, either in, or adsorbed by, the red blood corpuscles, and which causes the symptoms seen in bad superficial burns, and in some cases death.

3. Extracts of burned skin are toxic. Extracts of skin burned post-mortem are innocuous.

4. There is increased protein catabolism in burns as evidenced by the increase in the nonprotein nitrogen and urea nitrogen of the blood.

5. The contents of burn blisters are not toxic.

6. Whole citrated blood from burn cases is toxic. The blood serum is not toxic. Solutions of the corpuscles properly treated are more toxic than whole blood.

7. The toxic substance consists of two portions, one of which is thermostabile, diffusable and neurotoxic; the other is thermolabile, colloidal and necrotoxic. Chemically the toxin consists of primary and secondary proteoses.

8. No evidence of the production of antibodies against the burn toxin could be found.

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THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS*

I. The Effect of Benzyl Alcohol, Benzyl Acetate and Benzyl Benzoate when Given by Mouth upon the Blood Pressure, Pulse and Alimentary Canal

By Charles M. Gruber, Ph.D., M.D., St. Louis, Mo.

OPIUM and its alkaloids have been extensively studied for the past half century. The early works dealt mainly with their narcotic and toxic actions. During the past 25 years their actions on tissues other than that of the central nervous system have been investigated. As early as 1880, Salvioli[1] noted that perfused intestinal strips responded by increased force of contraction when tincture of opium was added to the perfusion fluid. Pal[2] in 1900 published the first paper of his series in which he demonstrated that opium decreased the tonus of the intestine and that morphine increased it. Two years later he[3] demonstrated that morphine, codeine, thebaine, apomorphine, apocodeine, heroine and dionine increase the tonus and the strength of the peristaltic movements of the intact intestine. He noted that the pure phenanthrene drugs (morpholin and other phenanthrene derivatives) did not produce the characteristic action of the pyridin-phenanthrene alkaloids. Eleven years later he[4] showed experimentally that papaverine and narcotine antagonized the action of muscarine on the intestine and bronchial musculature in guinea pigs, cats and dogs. He concluded that there are present in opium two groups of alkaloids: (1) the isoquinolin group, papaverine and narcotine—which decreases the tonus of the intestine and relaxes broncho-spasm, and (2) the phenanthrene group—morphine, codeine, etc.,—which has the opposite action. During the same year he[5] demonstrated that papaverine decreases the tonus of the urinary bladder and the uterus when the tonus of these organs was increased by pituitrin. He found this drug also to relax the gall bladder and the smooth muscle of the vessel walls —fall in blood pressure. He believed that papaverine is of value in the treatment of (1) bronchospasm (asthma), (2) intestinal colic, (3) hyperemesis gravidarum, (4) diarrhea, (5) hypertension.

Pal's results upon the intestine have been corroborated by a number of authors, viz., Popper,[6] Popper and Frankl,[7] Shapiro,[8] Zunz and György, Pancoast and Hopkins,[10] Uhlmann and Abelin,[11] Macht[12] and others. Popper[6] found morphine to increase the tonus of the intestine and pantopon to decrease it. Popper and Frankl[7] and Popper[13] demonstrated that the alkaloids of the phenanthrene group, morphine, thebaine, and codeine, increase the tonus and the force of peristaltic movements, whereas the alkaloids of the isoquinolin group, papaverine, narcotine, and narceine, decrease them.

*From the Department of Pharmacology, Washington University School of Medicine.

The latter author concludes that papaverine is valuable in the treatment of pertussis and pylorospasm. Pal's findings on the action of morphine upon the uterus were confirmed by Barbour and Copenhaven[14] and Barbour.[15] Jackson[16] confirmed and extended Pal's observations on the action of the opium alkaloids upon the bronchial muscles and upon the urinary bladder.[17]

Like Pal[5] Lieb and McWhorter[18] and Macht[19] showed that morphine contracted and papaverine relaxed the gall bladder.

In a series of researches Macht[20] studied the chemical structure of the opium alkaloids and their physiologic actions on smooth muscle. He found the pyridin group to produce contraction of smooth muscle. He noted that the alkaloids which contained the double nucleus, combination of isoquinolin and benzyl components, e.g., papaverine, produced relaxation of tonus and inhibition of the contractions of this muscle. On the contrary, those alkaloids possessing an isoquinolin nucleus alone did not inhibit the contractions, or relax the tonus, but stimulated them. He concluded that the benzyl component of the alkaloid was the portion active in depressing these tissues. In the same year he[21] studied the effect of benzyl benzoate and benzyl acetate upon smooth muscle. He noted that subcutaneous, intramuscular, intraperitoneal or intravenous administration of benzyl benzoate, benzyl acetate and benzyl alcohol inhibited rhythmic contractions lowered tonicity and relaxed spasms of these muscles. These drugs also produced a fall in blood pressure due to their action upon the lining of the vessel wall.

Upon the basis of his results, obtained with experimental animals and from clinical observations he felt that the benzyl esters might be of therapeutic value where there is spastic contraction of smooth muscle as in: (1) diarrhea with excessive peristalsis; (2) dysentery; (3) intestinal colic or enterospasm; (4) pylorospasm; (5) mucus colitis; (6) spastic constipation; (7) spastic conditions of gall bladder and biliary ducts; (8) spasmodic dysmenorrhea; (9) spastic contractions of the seminal vesicles; (10) ureteral colic; (11) vesical spasm; (12) enuresis due to hyperirritable bladder or sphincter; (13) angiospasm and hypertension of the circulatory system; (14) bronchospasm (asthma, pertussis, etc.).

Two years later he[22] reported favorable results with the use of benzyl benzoate clinically in circulatory hypertension and angina pectoris. He found that 25 minims of a 20 per cent alcoholic solution given by mouth three times a day reduced both systolic and diastolic pressures. Vasodilatation begins within 30 minutes after administration of the first dose and continues as long as, or longer than, that produced by a clinical dose of sodium nitrite. After repeated doses, the pressure may remain low several days, although the drug is withdrawn. He found it to have no deleterious effect upon the heart and kidneys.

Mason and Pieck[23] noted that an intravenous administration of a sufficiently large dose of benzyl benzoate is followed by a prompt, pronounced and prolonged fall in blood pressure. These changes they believe are due to the action of the drug on the heart.

Nielsen and Higgins[24] in a series of papers reporting their results upon

intravenous administration of benzyl compounds support the findings of Macht.

Upon scrutinizing the various records presented in these papers one is struck by the fact that in a large proportion of the animals it was necessary to resort to artificial respiration to maintain life after the desired results upon the intact organs were obtained. Macht[21] presents two curves in which respiratory paralysis as well as fall in blood pressure resulted. In nearly all cases in which relaxation of smooth muscle resulted after the administration of benzyl benzoate, Mason and Pieck[23] found the dose to be so large that respiratory paralysis occurred simultaneously with the relaxation. Nielsen and Higgins[24] show no less than 10 curves in which artificial respiration was necessary to revive the animal after intravenous administration of benzyl compounds.

Inasmuch as benzyl alcohol, benzyl benzoate and benzyl acetate are only slightly soluble in the blood serum it would seem that intravenous administration of these drugs would be accompanied by undesirable side actions, chief among them being the formation of emboli. Macht[22] has been able to reduce blood pressure clinically by oral administration as well as, in experimental animals, by intravenous, subcutaneous, intramuscular or intraperitoneal administration. This would lead one to believe that if there is formation of emboli it is of little consequence.

A number of questions in this connection have occurred to me: (1) Do benzyl alcohol and its esters, benzyl benzoate and benzyl acetate, produce a fall in blood pressure in dogs when given orally by stomach tube? (2) How does this change in blood pressure compare with that obtained by the nitrites similarly administered? (3) What effect have these substances on the pulse rate when given by mouth? (4) What effect, if any, have these esters upon the alimentary canal when given by this route?

METHOD

The blood pressure was determined both by the indirect and direct methods. The indirect method of determining the blood pressure in the unanesthetized dog was the same as that used by Kolls.[25] The apparatus was made according to the specifications presented by him in his article except that a "Bakelite" barrel was substituted for the brass barrel on the piston recorder and a long roll kymograph for the short one.

The criteria employed in the estimation of the systolic and diastolic pressures in the indirect method were those customarily used. These results were controlled by the Korotkoff sounds[26] which were followed with a stethoscope.

Control experiments quickly demonstrated that a possible error of as much as 25 per cent could exist if the cuff, when once removed, was not replaced over the same area of the dog's leg. In all instances, therefore, before the metal cuff was removed its upper and lower edges were marked with ink upon the dog's limb. When it was replaced, utmost care was employed to see that it occupied the same position as before and that it fitted

equally snug in each case. The error was thereby reduced to less than 5 per cent.

The dogs used in these experiments weighed from 21 to 27 kilos. and were unanesthetized. They were healthy animals less than two years old and had been kept in the laboratory for more than six months, during which time they were petted and trained to permit the author to apply the cuff upon the left hind limb and to increase the pressure within the cuff to 250 mm. of mercury without their offering resistance or being curious. Two of these dogs found this manipulation of so little interest and inconvenience that they would voluntarily leap upon the table and lie down and would sleep while the experiments were in progress. Some of the animals showed no dislike to the passage of the stomach tube and one would swallow erythrol tetranitrate tablets after these were placed on his tongue.

The animals were not fed the morning of the experiment so that in every case the stomach contained no food. The drug administered by stomach tube was washed down with 20 to 50 ml. of water to assure the passage of the entire amount into the stomach.

These animals were kept quiet for 15 to 20 minutes, after which several readings of the blood pressure were taken before the drug was administered. During the experiment, also, the animal was kept quiet, lying on its right side except under certain unavoidable conditions of which I shall speak later. The animals would frequently submit to this as long as six hours with practically no movement. Blood pressure readings were taken at intervals of from 15 minutes to 1 hour when the benzyl esters were tested, but every 2 to 5 minutes when drugs of the nitrite series were given. The results thus obtained were calculated in mm. of mercury and plotted in curves.

Smaller dogs weighing from 4 to 8 kilos. were used in those experiments in which the direct method of determining blood pressure was used. They were anesthetized with either urethane (2 grams per kilo. body weight by stomach) or with paraldehyde (1.8 ml. per kilo. body weight by stomach) supported by ether. A cannula was placed in the left carotid artery and connected to a membrane manometer by heavy rubber tubing after the entire system was filled with sodium carbonate solution as an anticoagulant. A chronometer marking the time interval, usually in minutes, was placed at the zero blood pressure level. The writing surfaces were adjusted so that they wrote vertically one above the other on the smoked drum of a slowly moving kymograph. The preliminary procedures as to feeding, etc., were the same as with the other animals. After the stomach was washed with lukewarm water the drug was given in the manner described.

The dogs used in those experiments in which the action of the benzyl esters was tested upon the alimentary canal varied in weight from 6 to 27 kilos. These were used unanesthetized and had not had food for about 15 hours. The drug was given by stomach tube and in all instances followed by 20 to 50 ml. of water. The animals were then turned loose in their runways and watched.

A number of benzyl preparations were used in the course of this work. They were obtained from the following firms: benzyl benzoate from Hynson, Westcott & Dunning and from the Eastman Kodak Company; benzyl acetate from the Abbott Laboratories and from Kahlbaum; and benzyl alcohol from Merck, and from Hynson, Westcott & Dunning. The benzyl benzoate was used in both the pure form and in 20 per cent alcoholic solutions while the benzyl acetate was used in the pure form and dissolved in Squibb's olive oil. The benzyl alcohol was used pure.

Ethyl alcohol was used in 95 per cent solution diluted by the water used in washing the stomach tube free from alcohol. Erythrol tetranitrate was administered in ½ grain tablets compounded by Merck for clinical purposes. U. S. P. preparations of sodium nitrite and spirits of nitroglycerin were employed in 0.2 gm. doses for the former and in 2 to 6 minim doses for the latter.

Results

The results of the blood pressure determinations by the indirect method are somewhat higher than those usually found in the dog by the direct method. Kolls[27] and Erlanger[28] found in their experiments that there was little difference in the pressures obtained by the two methods when taken simultaneously. In this work the systolic pressure varied from 173 to 200 mm. of mercury. Kolls presents a record from an unanesthetized dog of 190 mm. Erlanger's average, taken from three anesthetized dogs by the indirect method after the limb was converted by operation into a cylinder, was 200 mm. In my experiments the diastolic pressure determinations varied from 77 to 105 mm. of mercury. In Kolls' record the diastolic pressure was 120 mm. Erlanger's average was 125 mm.

I. The Effect of Benzyl Alcohol and its Esters, Ethyl Alcohol and the Nitrite Group upon Blood Pressure

Benzyl Alcohol.—Benzyl alcohol was given in doses ranging from 0.1 ml. to 1.0 ml. per kilo. body weight. In no instance, either in the normal unanesthetized animal or in those anesthetized, was the blood pressure lowered. The effect of a small dose, 0.1 ml. per kilo. (2 ml. total dose for 21 kilo. dogs), upon the blood pressure in unanesthetized animals can be seen in Charts I and II in which the data from two animals are plotted. In Chart I, curve (1) after the drug had been administered, the systolic blood pressure varied from 200 as the maximum to 190 as the minimum. The control (or reading before the drug was given) was 192 mm. of mercury. The diastolic control was 80 mm. of mercury. After the administration of the drug this pressure varied for the 15 readings from 87 as the maximum to 79 as the minimum.

In Chart II, curve (1) a similar variation was observed. The control reading for the systolic pressure was 173 mm. of mercury. The thirteen determinations after the administration of the dose varied from 177 as the maximum to 170 as the minimum. The diastolic pressure with the control at 77 mm. of mercury varied from 83 as the highest to 73 as the lowest. These variations in (maximum and minimum) pressures are, I believe, well within the limit of error.

The effect of a larger dose (0.2 ml. per kilo, total of 5.2 ml., upon an animal weighing 27 kilos.) can be seen in Chart III, curve (1). In this animal the systolic pressure was 190 mm. of mercury at first but was increased by benzyl alcohol to 201 mm. Later it fell to 185 mm. The minimum diastolic pressure was 92 mm., the maximum 102 mm., the control 97 mm. of mercury. In this as in all other experiments in which moderately large doses were used a rise in blood pressure was obtained. This rise was probably not due to the direct action of the drug upon the circulatory system but indirectly through the restlessness of the animal caused by the cathartic and

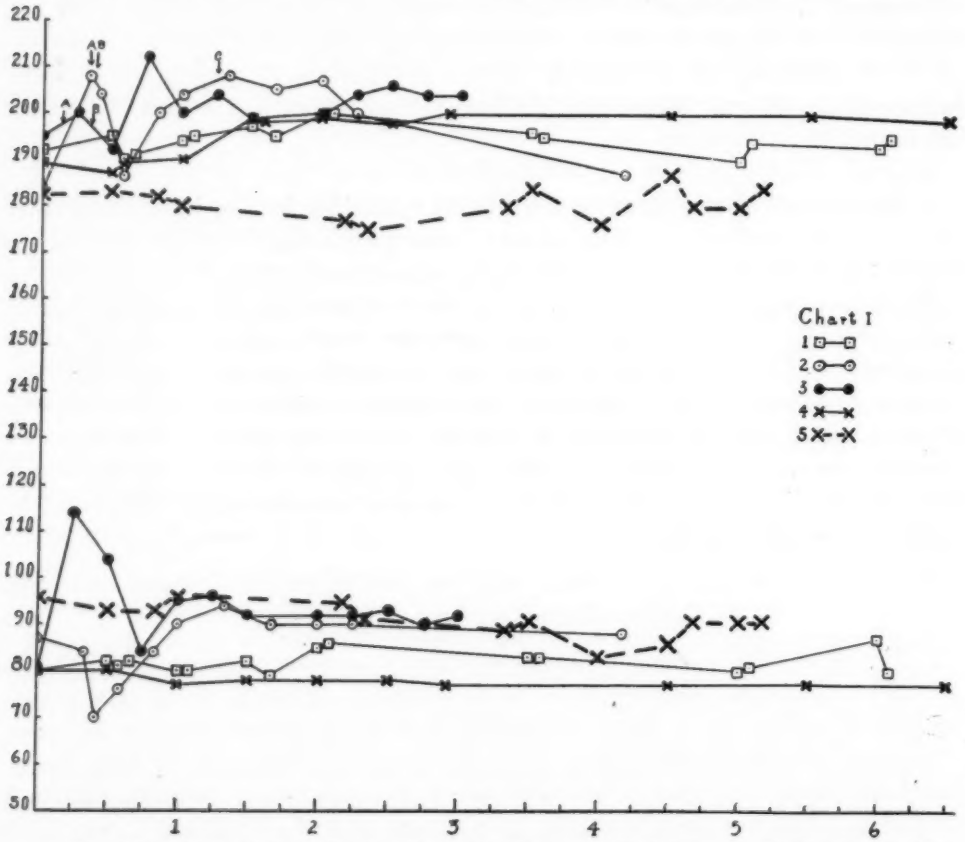


Chart I.—This and the following three charts were plotted from the blood pressure data obtained from three unanesthetized dogs with the use of the indirect method as described by Kolls. The curves in each chart were plotted from the data of one animal. The laboratory procedure was that described in the text. Upper curves, systolic blood pressures; lower curves, the diastolic pressures. The blood pressures are plotted on the ordinate against the time in hours on the abscissa.

Dog C, weight 21 kilos. *1*, two ml. pure benzyl alcohol; *2*, 4.2 ml. pure benzyl acetate in olive oil; *3*, 8.4 ml. pure benzyl acetate in water; *4*, 11.2 ml. pure benzyl benzoate; *5*, 21 ml. pure benzyl benzoate. *A*, animal restless; *B*, salivated; *C*, defecated.

emetic effects of the drug. It was impossible to keep the animal quiet because of the irritating action of the substance upon the alimentary canal. This action was so marked that the animal defecated twice (at c) before the experiment was concluded. In other animals borborygmi were heard and the animals would resist all petting and jump from the table to vomit or defecate. In the anesthetized animals as in those unanesthetized benzyl

alcohol had no effect upon the blood pressure when given by stomach tube.

Benzyl Acetate.—Like benzyl alcohol, small doses of benzyl acetate, 0.1 ml. per kilo., had no effect upon blood pressure when given orally in either unanesthetized or anesthetized animals. Doses of 0.2 ml. or more per kilo. produced a rise in blood pressure because the drug irritated the alimentary canal to such an extent that the animals could not be kept quiet. Chart I, curves (2 and 3) were plotted from the data of two such experiments upon the same animal. In curve (2) the animal received 0.2 ml. of pure benzyl acetate diluted with olive oil; in curve (3) 0.4 ml. per kilo. diluted with

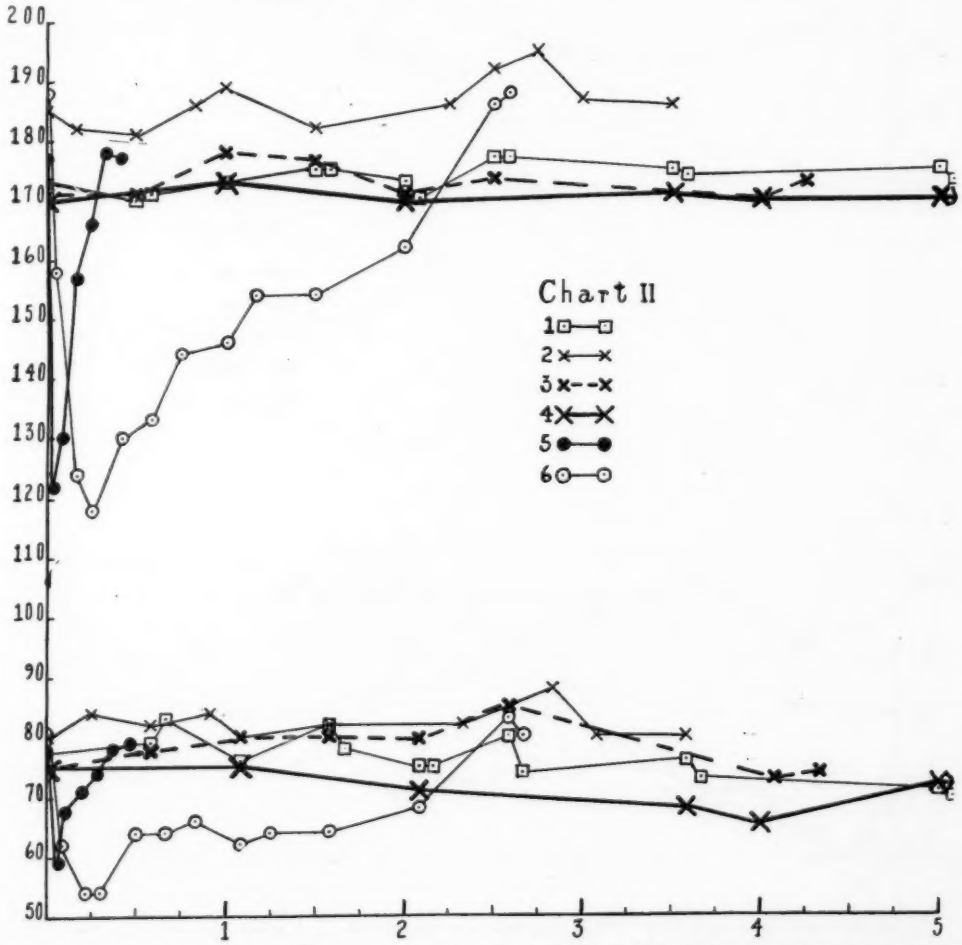


Chart II.—Dog B, 21.5 kilos. *1*, two ml. pure benzyl alcohol; *2*, two ml. benzyl benzoate, 20 per cent by volume in alcohol; *3*, 10.8 ml. pure benzyl benzoate; *4*, 21 ml. pure benzyl benzoate; *5*, 6 minims spirits of nitroglycerin; *6*, 0.2 grams sodium nitrite.

water. In both experiments borborygmi were heard, the animal became restless and salivated and defecated before the experiment could be concluded.

The direct readings upon the anesthetized animals confirm the indirect determinations. In no experiment was a fall in blood pressure seen. This drug was also tried upon one cat with similar results.

Benzyl Benzoate.—Unlike benzyl alcohol and benzyl acetate, benzyl benzoate possesses very little irritating action on the alimentary canal. Except in two dogs large doses of pure benzyl benzoate, 1 ml. per kilo., were given without provoking vomiting and there was only a mild cathartic action. This substance seems to possess some narcotic action. After being given

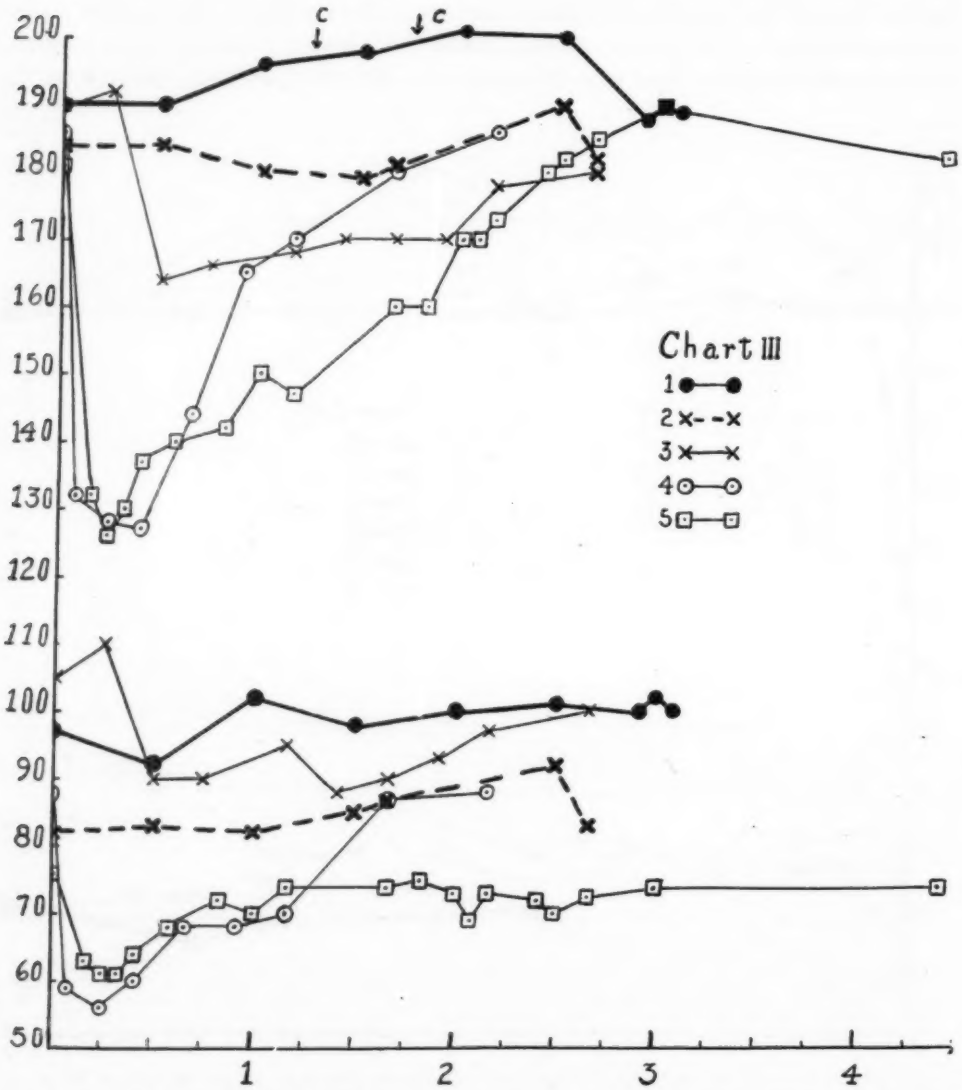


Chart III.—Dog L, weight 27 kilos. *1*, 5.2 ml. pure benzyl alcohol; *2*, 14 ml. pure benzyl benzoate; *3*, 10.4 ml. benzyl benzoate, 20 per cent by volume in alcohol + 8 ml. ethyl alcohol, + 20 ml. water; *4*, 18 ml. ethyl alcohol 95 per cent + 3 ml. olive oil + 20 ml. water; *5*, 18 ml. 95 per cent ethyl alcohol + 100 ml. water.

large doses, 1 ml. per kilo., of pure benzyl benzoate the dogs appeared to be sleepy and would lie on the table quietly for as long as 6 hours. Usually when these animals were turned back into their runways they would have oily, liquid stools with somewhat the odor of benzyl benzoate. Benzyl ben-

zoate was also given in amounts considered to be the therapeutic dose for humans, 2 ml. 20 per cent in alcoholic solution.

Charts II and IV, curves (2) and (1) respectively were plotted from the data of two experiments. In Chart II, curve (2) some variation is found in the blood pressure record. This is due to the restlessness of the animal. In Chart IV, curve (1) the blood pressure remained at almost a constant level for more than 4 hours after the benzyl benzoate was given.

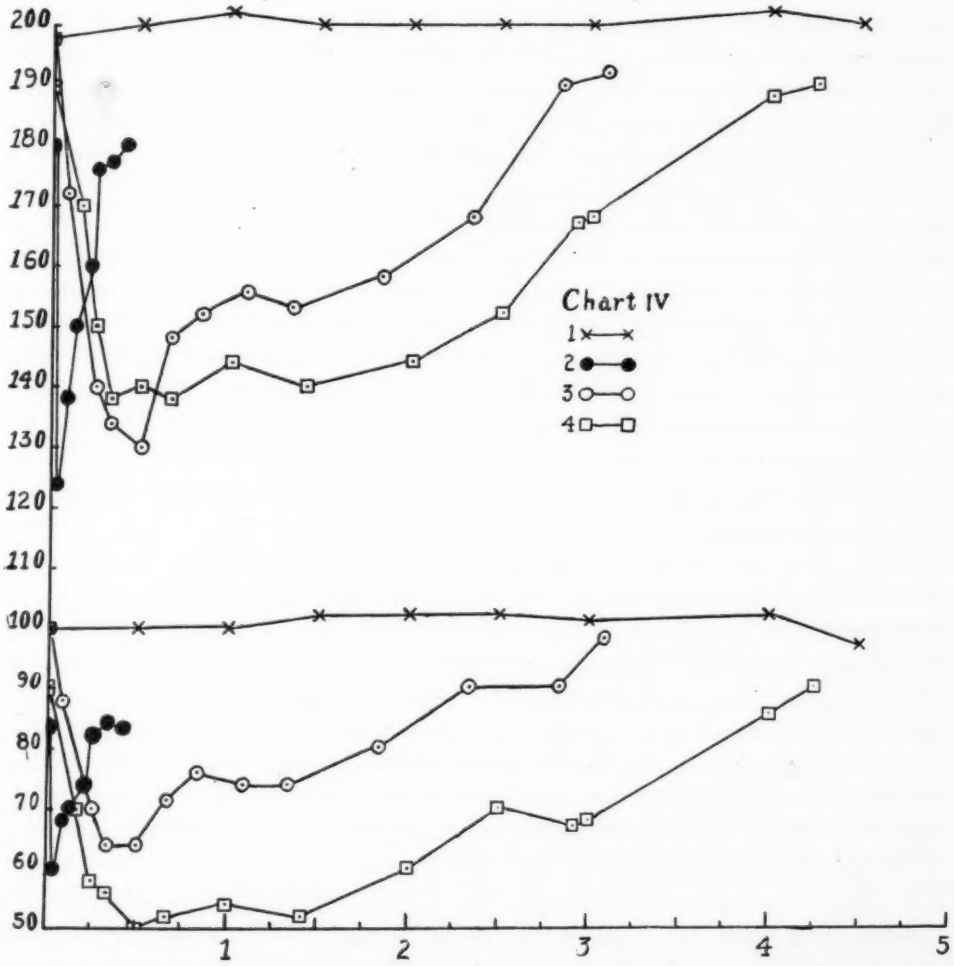


Chart IV.—Dog L, weight 27 kilos. *1*, 2 ml. benzyl benzoate 20 per cent in alcohol by volume; *2*, 4 minims spirits of nitroglycerin; *3*, 0.2 grams sodium nitrite; *4*, 1 grain erythrol tetranitrate.

Upon experimentation it was soon learned that larger doses of the alcoholic solution of benzyl benzoate could not be given without taking into consideration the action of the alcohol itself. Its effect, however, was not constant for the different animals. Some were much more susceptible to alcohol than others. That alcohol itself has an action upon blood pressure can be seen by comparing curves (2) and (3) with curves (4) and (5), in Chart III. Curve (2) is the data obtained from the animal after it had received 0.5 ml.

per kilo. of the pure benzyl benzoate, a total of 14 ml. The blood pressure changed very little. The control systolic pressure was 183 mm. of mercury. This reached a minimum of 179 mm. in 1½ hours, while the diastolic pressure did not drop below the control level. The fall in systolic pressure is within the error of experimentation. In curve (3) taken from the same animal, 10.8 ml. of alcoholic solution of 20 per cent benzyl benzoate, 8 ml. ethyl alcohol and 20 ml. of water were given. The systolic blood pressure fell within 30 minutes from 190 to 163 mm. of mercury (a fall of 29 mm.); the diastolic pressure from 105 to 90 mm. of mercury (a fall of 15 mm.). In curve (4) 18 ml. ethyl alcohol 95 per cent, 3 ml. Squibb's olive oil and 20 ml. of water were given, and in curve (5) 18 ml. ethyl alcohol 95 per cent, and 100 ml. of water were administered. In both experiments the animal became drowsy and remained motionless upon the table unless aroused. The systolic blood pressures dropped within 15 minutes from 186 and 182 mm. to 128 and 126 mm. of mercury (58 and 56 mm.) while the diastolic pressures dropped from 88 and 76 to 56 and 62 mm. of mercury respectively.

Large doses of benzyl benzoate have no effect upon blood pressure in the dog when given by mouth (see Charts I and II and Fig. 1). In Chart I, curve (4), 11.2 ml. and in curve (5), 21 ml. of pure benzyl benzoate were administered to a dog weighing 21 kilos. A slight systolic fall was noted with the larger dose, 7 mm. of mercury, or 4 per cent which is less than the possible experimental error.

In Chart II, curve (3), 10.8 ml. and in curve (5), 21.2 ml. of pure benzyl benzoate were given to another animal weighing 21.5 kilos. In neither instance was a fall in blood pressure noted. In both animals the stomachs were washed 4 hours after the drug had been given but no benzyl benzoate recovered.

Benzyl benzoate like benzyl alcohol and benzyl acetate when given by stomach tube, has practically no effect upon the blood pressure in the normal, unanesthetized dog. This is true even with huge doses, 1 ml. per kilo. body weight. Calculated on the basis of 0.1 ml. pure benzyl benzoate (25 to 30 minims of a 20 per cent alcoholic solution) as the clinical dose for man weighing 150 pounds or 68 kilos., these animals received 210 clinical doses or about 630 times as much as that given to man per kilo. body weight.

These observations are supported by the experiments performed upon anesthetized animals in which the benzyl benzoate was given similarly by mouth, but the blood pressure recorded by placing a cannula in the left carotid artery (see Fig. 1). The animal in this experiment weighed 5.7 kilos., and received 2 grams of urethane per kilo. by stomach 1½ hours before the experiment was begun, and ether during the experiment. At (1), the stomach having been washed, 7 ml. pure benzyl benzoate, 1.2 ml. per kilo. body weight, were given before the tube was withdrawn. The blood pressure at this time was 95 mm. of mercury. After 1 hour there was no change in the blood pressure. In 2 hours at (2) the blood pressure had mounted to 110 mm. of mercury. The stomach was then washed out and 0.2 grams of sodium nitrite given in a 2 per cent solution which was further diluted by

20 ml. of water. In 40 minutes the blood pressure had gradually dropped to 80 mm. of mercury, or a fall of 30 mm. At (3) 5 minims spirits of nitroglycerin were dropped upon the animal's tongue. Within two minutes the blood pressure dropped 20 mm. of mercury.

II. DEPRESSOR ACTION OF THE DRUGS OF THE NITRITE SERIES

As a check upon the methods used in the above experiments spirits of nitroglycerin, sodium nitrite and erythrol tetranitrate were given in clinical doses to the same dogs that had received the benzyl esters. In every instance a profound fall in both systolic and diastolic pressures resulted. These results are illustrated in Charts II and IV and Figs. 1 and 2.

Spiritus Glycerylis Nitratis.—In Chart II, curve (5), an animal weighing 21.5 kilos. was given 6 minims of spirits of nitroglycerin upon the tongue. The systolic blood pressure within 2 minutes dropped from 177 to 122 mm. of mercury, a fall of 55 mm. and the diastolic pressure fell simultaneously from 78 to 59 mm., or 19 mm. of mercury. Within 15 minutes both systolic and diastolic pressures had returned to normal. In Chart IV, curve (2), the dog weighing 27 kilos. was given 4 minims of spirits of nitroglycerin dropped upon the tongue and within two minutes the systolic blood pressure had dropped from 180 to 124 mm., a fall of 56 mm. and the diastolic pressure from 84 to 60 a fall of 24 mm. of mercury. Within 25 minutes the blood pressure was again normal.

Sodium Nitrite.—Like spirits of nitroglycerin sodium nitrite produced a prompt fall in both systolic and diastolic pressures in unanesthetized animals. See Chart II, curve (6) and Chart IV, curve (3) and Fig. 2. In Chart II, curve (6) was taken from an animal weighing 21.5 kilos. which had been given by stomach tube 0.2 grams of sodium nitrite in a 2 per cent solution followed by 20 ml. of water. In 15 minutes the systolic blood pressure had dropped from 188 to 118, or a fall of 60 mm. of mercury. The diastolic pressure simultaneously fell from 81 to 54 mm., a fall of 27 mm. of mercury. Two and a half hours after the administration the blood pressure was back to its control height. Fig. 2 is a graphic record from the data of the same animal showing the indirect readings in blood pressure. The systolic blood pressure fell within 15 minutes from 183 to 118 mm. (65 mm.) and the diastolic fell from 77 to 34 mm. (43 mm.). The effect on pulse rate can also be seen.

In Chart IV, curve (3), a dog weighing 27 kilos. was given 0.2 gram of sodium nitrite in a 2 per cent solution by stomach tube, followed by 20 ml. of water. The systolic blood pressure in 30 minutes had fallen from 198 to 130 mm. of mercury (68 mm.) while the diastolic simultaneously fell from 100 to 64 mm. of mercury (36 mm.). Three hours after the administration the blood pressure was back almost to its control height.

Erythrol Tetranitrate.—The action of this drug upon the unanesthetized dog is well represented in Chart IV, curve (4). In this instance the dog was given 1 grain of erythrol tetranitrate in 40 ml. of water by stomach tube. In 20 minutes the systolic blood pressure had dropped from 190 to 138 mm. of mercury, a fall of 52 mm. Simultaneously the diastolic blood

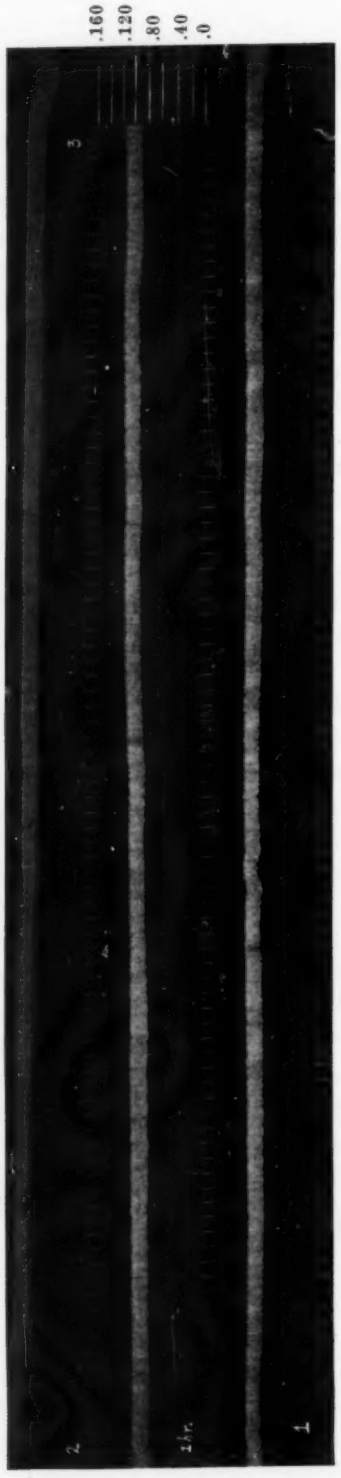
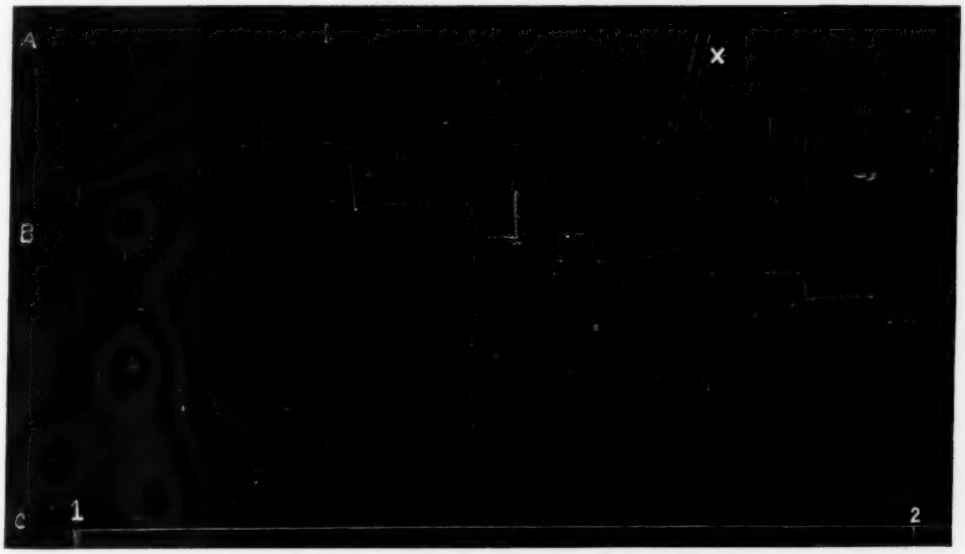


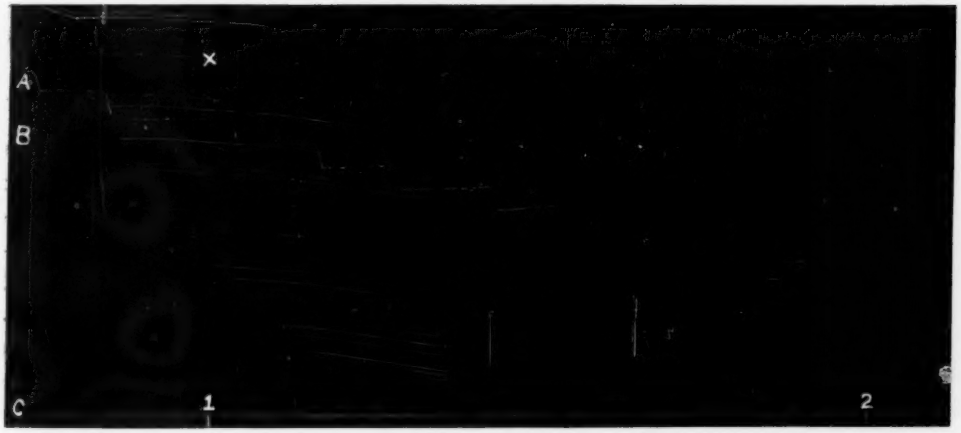
Fig. 1.—Dog, 5.7 kilo. Urethane anesthesia supported by ether. Time in 30 seconds and zero blood pressure. *1*, 7 ml. pure benzyl benzoate; *2*, two hours after benzyl benzoate was given 0.2 gram sodium nitrite was administered; *3*, spirits of nitroglycerin 5 minims. Calibration of membrane manometer on figure.

pressure fell from 90 to 56 mm. of mercury, but it dropped 6 mm. more during the next 10 minutes so that the total fall was 40 mm. Both systolic and diastolic blood pressures had returned to the control level 4¼ hours after the administration of the drug.

The author found that not all dogs respond equally well to the nitrite group of drugs. In some instances the fall in blood pressure amounted to



A Curve I.



B Curve II.

Fig. 2.—Dog B, 21.5 kilos. No anesthesia. Animal given 0.2 grams of sodium nitrite after curve I was recorded. Curve II taken 15 minutes after the drug was given. *X*, animal contracted muscles in limb; *A*, pressure in mm. of mercury; *B*, pulse; *C*, zero blood pressure line and point of appearance of the Korotkoff sounds; *1*, systolic, *2*, diastolic pressure.

less than 20 mm. of mercury. In others the fall was slightly more, but of brief duration. Dogs L and B responded very quickly with a fall in blood pressure to all the drugs of the nitrite series and also to ethyl alcohol.

III. THE EFFECT OF BENZYL ESTERS AND NITRITES WHEN GIVEN ORALLY UPON THE PULSE RATE IN NORMAL, UNANESTHETIZED DOGS

Mason and Pieck[23] found that benzyl benzoate produces marked slowing of the heart when injected intravenously. They believe that this effect is the essential, if not the only, factor in producing a fall in blood pressure.

In this work an attempt was made to determine if the benzyl esters have an effect upon the pulse of the normal unanesthetized animal when

TABLE I

A TABLE SHOWING THE EFFECT OF (1) PURE BENZYL BENZOATE; (2) TWENTY PER CENT BENZYL BENZOATE IN ALCOHOL; (3) PURE BENZYL ALCOHOL; (4) REST UPON THE PULSE RATE IN THE SAME UNANESTHETIZED DOGS. PULSE RATE PER MINUTE.

DRUG	DOSE IN ML.	DOG	CONTROL RATE		TIME AFTER ADMINISTRATION OF DRUGS IN HOURS.									
					½	1	1½	2	2½	3	3½	4	4½	5
1	14.0	L	85		70	70	70							
	10.0	B	60	60	55	55		50	55	55				
	10.8		80	75	60	60	55	55		55	55			
	21.0		70	80		70		50			45	50		50
	21.0		100	100	80	70	70	50	70		50			
	11.2	C	65		65	60		55		45			55	
	11.0		85		80	55		50	55	50	55			
	21.0		50	50	50	40		40	40	45		45	45	50
	21.0		70	60		60		55	45		45	45		50
2	2.0	L	95	85	65	85	65	60						
	2.0	B	65	60	65	60	55		45		50	50		
	2.1	C	85	75	70	50	45							
3	5.4	L	70		70	75	72	70		65				
	2.0	C	90	80	55	45	50	45	45	55		50		
	4.2		85	85	65	60	60	60	60	60	60			
4		L	90		71	66	48	52						
		B	90			80	63	59	66					
			88		82	70	64	60	66					
		C	96			84			68	50	65			
			90		86	58	56	44	48					

given by stomach tube. Table I shows the effects of benzyl benzoate 20 per cent alcohol solution, pure benzyl benzoate, benzyl alcohol and of rest upon the pulse in the same animals. The table seems to show that the pulse is slowed by the administration of benzyl alcohol and benzyl benzoate. However, a comparison of these results with those obtained with rest alone would suggest that this slowing can be accounted for by the fact that the animals are resting throughout the experiments. In most instances both when drugs are administered and with rest alone the maximum slowing of the pulse occurs 2 or 3 hours after the beginning of the experiments. Similarly the marked slowing of the pulse after large doses of benzyl benzoate is due to rest in addition to the slight narcotic action of the drug on the animal. The slowest rates were obtained in all those experiments in which the animal slept during the interval in which the pulse rate was being determined.

The drugs of the nitrite series produced marked acceleration of the pulse rate in the unanesthetized dog. See Table II. This acceleration is due not to the action of these drugs upon the heart muscle but to the fall in blood pressure[29] and depression of the cardio-inhibitory center[30] and pos-

sibly to some excitation of the accelerator center.[31] The same factors are probably concerned in the slight acceleration of the pulse rate after the administration of a moderate dose of ethyl alcohol.

IV. THE ACTION OF BENZYL ALCOHOL AND ITS ESTERS UPON THE ALIMENTARY CANAL WHEN ADMINISTERED BY STOMACH TUBE

In all the experiments reported in which the action of the benzyl esters was tested upon smooth muscle (intestine) the drugs were given either

TABLE II

A TABLE SHOWING THE EFFECT OF (1) ETHYL ALCOHOL 95%; (2) SPIRITS OF NITROGLYCERIN; (3) SODIUM NITRITE; (4) ERYTHOL TETRANITRATE UPON THE PULSE RATE IN UNANESTHETIZED DOGS. PULSE RATE PER MINUTE.

DRUG	DOSE	DOG	CONTROL RATE		TIME AFTER ADMINISTRATION OF DRUGS IN MINUTES									
					2	5	10	15	30	60	90	120	150	180
1	18 ml.	L	90	80			100	100	100	80		80		
	18 ml.	L	75	65			85	75	75	80	75	65	60	60
	20 ml.	B	75	95				95	105	120	75	75	65	60
2	4 m	L	80		140	100	80	75	80					
	6 m	B	80	65	120	110	100	90	80					
	4 m	C	50	45	80	70	70		60	50				
3	0.5 gm.	L	90	90		110		100	110	95				
	0.2 gm.	L	60	65		80		90	85	80		100		65
	0.2 gm.	B	65	60		125		140	120	135	140	105	80	80
	0.25 gm.	C	75	65				110	110	110	90	80		
4	1 grain	L	105	95			105	95	85	85	85	75	55	50
	1 "	C	75	60			60		50	80	55			
	1½ "	C	75	50			80		80	50	50			
	2 "	C	60					75	90	60		50		

intravenously or added to the solution in which the excised strip of intestine was contracting[21, 23, 24]. Macht[32] discarded the use of benzyl acetate in therapeutic practice because, he says, "its sharp banana-like odor was found to produce discomfort or indigestion." He[33] makes no note of the action of massive doses of benzyl esters on the digestive system of the unanesthetized animal used in studying the toxicology of these substances. He states, "In dogs, benzyl esters can be administered by mouth without toxic effects almost *ad libitum*; thus as much as 2 ounces or 70 c.c. of pure benzyl benzoate in one dose have been introduced into the stomach of a dog weighing 10 kilo. without any harmful effects."

Nielsen and Higgins[24] fed a dog weighing approximately 4.5 kilos. ½ ounce of benzyl cinnamate without its showing any noticeable symptoms.

I found that large doses of benzyl alcohol and benzyl acetate could not be used in studying their effect upon blood pressure in dogs not anesthetized because of their disquieting effect upon the alimentary canal. In two animals only, huge doses of pure benzyl benzoate produced irritation, vomiting and diarrhea. In all other animals the result was an oily, liquid stool after the animals were returned to their runways.

The brief summary of the protocols here presented indicates that moderate doses of benzyl acetate and benzyl alcohol possess emetic and cathartic

TABLE III

IN THIS TABLE IS PRESENTED A BRIEF SUMMARY OF THE PROTOCOLS OF THE EXPERIMENTS PERFORMED. SMALLER DOSES WERE GIVEN BY STOMACH TUBE, BUT THESE HAD NO EFFECT AND ARE NOT INCLUDED IN THE TABLE. SUBCUTANEOUS AND INTRAMUSCULAR INJECTIONS ARE INDICATED BY THE (*).

DOG	DRUG			QUANTITY ML.	WEIGHT OF ANIMAL IN KILOGRAMS	TIME OF ADMINISTRATION	VOMITED	DEFECATED
C	Benzyl alcohol		(M.)	10.5	21.0	2:05	2:15 2:18 2:27 2:35 2:36	2:38 2:39
S	"	"	(H.W.D.)	2.5	12.5	8:50	8:53	——
L	"	"	(H.W.D.)	5.2	27.0	2:05	3:10	3:15
S	"	"	(H.W.D.)*	6.0	12.5	11:19	——	——
S	"	"	(H.W.D.)	6.0	12.5	3:32	3:39 3:43 3:50 3:58 3:59	3:42 3:48 3:55
B	"	"	(M.)	10.8	21.5	1:38	1:48 1:52 1:59	1:51
C	Benzyl acetate		(K.)	4.2	21.0	9:40	——	10:39
C	"	"	(A.)	11.0	21.0	1:42	1:55 1:56	2:07
C	"	"	(A.)	18.	21.0	2:00	2:26	2:14
L	"	"	(K.)	7.9	27.0	8:45		10:37
L	"	"	(K.)	13.2	27.0	9:00	9:41 9:42	9:27
L	"	"	(A.)	14.	27.0	1:44	2:40	2:15
S	"	"	(K.)	6.1	12.5	1:55	2:00 2:20	——
S	"	"	(K.)	6.1	12.5	9:10	9:22 9:32 9:40 10:18	9:20 9:29 9:30 9:35 10:37
S	"	"	(K.)*	5.0	12.5	8:35	——	——
S	"	"	(A.)	5.0	12.5	1:30	1:53 2:02 2:04 2:05	1:45 2:00
B	"	"	(A.)	10.1	21.5	1:30	1:54 2:17	——
B	"	"	(A.)	18.	21.5	2:02	2:12 2:13 2:21 2:26	2:16
J	"	"	(A.)	7.0	14.0	10:15	10:21 10:45	——
J	"	"	(A.)*	7.0	14.0	1:45	——	——
M	"	"	(K.)*	5.5	7.2	8:50	——	——
M	"	"	(K.)	5.5	7.3	12:54	1:35 1:42 1:50 2:00	12:57

TABLE III—CONT'D

DOG	DRUG	QUANTITY ML.	WEIGHT OF ANIMAL IN KILOGRAMS	TIME OF ADMINISTRATION	VOMITED	DEFECATED
A	" " (K.)*	4.5	6.0	8:45	——	——
A	" " (A.)	4.5	6.0	12:53	1:04	1:33
					1:12	1:34
						1:35
E	" " (A.)*	10.	9.	8:50	——	——
E	" " (A.)	10.	9.	1:25	4:20	4:50
					4:30	
F	" " (A.)*	7.	7.	8:55	——	——
F	" " (A.)	7.	7.	1:27	2:02	1:58
					2:20	1:59
G	" " (A.)*	8.	7.7	9:00	——	——
G	" " (A)	5.	7.7	1:30	3:30	4:00
					3:32	
					4:10	
D	Benzyl benzoate (A.)*	15.	14.	8:45	——	——
D	" " (A.)	15.	14.	1:15	3:10	2:19
					3:15	2:20
						2:21
S	" " (E.)	6.1	12.5	9:15	9:32	9:28
					9:50	
S	" " (E.)	14.1	12.5	3:25	4:00	——
					4:18	
					4:25	
					4:40	
					4:43	
S	Croton Oil in 5 ml. olive oil followed by 50 ml. water	4 drops	12.5	2:45	3:02	2:56
B	"	7 drops	21.5	2:50	——	2:59
						3:00
						3:22
B	"	0.6 ml.	21.5	1:27	2:01	2:02
C	"	0.5 ml.	21.0	1:28	——	1:56
						1:57
C	"	8 drops	21.0	9:32	9:40	10:45
					9:45	10:50

(M.) Merck's (K.) Kahlbaum
(H.W.D.) Hynson, Westcott & Dunning (A.) Abbott Laboratories
(E.) Eastman Kodah Company

properties. The time interval between the administration and the cathartic action is too short to be due to relaxation of the smooth muscle lining the alimentary canal. In some animals the irritation of the gut was so extreme, the peristalsis evidently so rapid, that the material passed by the bowel had the same appearance and odor as the vomitus. These drugs do not act upon the vomiting center in producing emesis but locally through irritation of the gastric and intestinal mucosa. Subcutaneous and intramuscular administration of these drugs in like quantities in these same animals produce neither emesis nor defecation. Benzyl alcohol was injected subcutaneously in only one animal. In this case the injection was followed by a "cold" abscess and sloughing of the entire area infiltrated.

From the above cited experiments I assume that the gastric discomfort or indigestion reported by Macht[32] and thought to be due to the "banana-like odor" was really due to the irritation of the gastric mucosa by the drug.

A few experiments in which croton oil was used are also included in the list of protocols. These experiments were performed on the same animals which received the benzyl compounds. The oil was diluted with olive oil and given by stomach tube. Smaller doses of croton oil than those listed were used, but these had no effect.

SUMMARY

1. Blood pressures were taken indirectly upon unanesthetized dogs and directly upon anesthetized animals which were given benzyl alcohol, benzyl benzoate, benzyl acetate and ethyl alcohol and drugs of the nitrite series by mouth.

2. No change in blood pressure was observed in dogs when benzyl alcohol, benzyl acetate or benzyl benzoate was administered orally. This result differs from the results of previous workers[21, 23, 24] in which the drugs were administered subcutaneously, intramuscularly, intraperitoneally or intravenously in the same experimental animal.

3. The drugs of the nitrite series lower both systolic and diastolic blood pressures in normal, unanesthetized dogs when given by mouth, the same as when given clinically in human beings.

4. Moderate doses of benzyl benzoate 20 per cent by volume in alcohol produce a fall in blood pressure. This fall is due, we believe, to the alcohol they contain and not due to the benzyl esters.

5. In susceptible, unanesthetized animals moderate doses of ethyl alcohol produce a fall in blood pressure.

6. When given orally benzyl alcohol, benzyl acetate and benzyl benzoate do not alter the pulse rate. The drugs of the nitrite series and ethyl alcohol cause acceleration of the pulse due to the fall in blood pressure and resultant anemia of the cardiac centers.

7. Benzyl alcohol and benzyl acetate possess emetic actions. This is also observed in some animals with benzyl benzoate. The action is due to local irritation of the alimentary canal and not due to excitation of the vomiting center. Subcutaneous and intramuscular injections of the same dose as given by mouth in the same animal do not produce emesis.

8. Moderate doses of benzyl alcohol and benzyl acetate and benzyl benzoate possess cathartic actions. This, however, is much more marked and of more drastic character with the two former substances in which profuse watery stools resulted. This action is due to the local irritation by the drug since (1) the time interval in most cases is too brief to be due to relaxation of the intestine, and (2) since subcutaneous and intramuscular injections are not accompanied by evacuation of the bowels.

9. Benzyl benzoate possesses a very slight narcotic action when given in huge doses, 1 ml. per kilo body weight, in the dog.

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SOME OBSERVATIONS ON TARTAR EMETIC*

By Walter G. Christiansen and Arthur J. Norton, Boston, Mass.

DURING the treatment of certain tropical diseases with tartar emetic, differences of opinion arose regarding the stability of tartar emetic solutions and the sterilization of these solutions. Some clinicians speak indefinitely about the appearance of precipitates in such solutions but can give no information as to the conditions under which they appear. To secure some exact data on this matter a specimen of potassium antimonyl tartrate was prepared very carefully and purified. The calculated antimony content of potassium antimonyl tartrate ($C_4H_4O_7KSb.\frac{1}{2}H_2O$) is 36.2 per cent and that found in our material is 36.3 per cent.[1]

Collins and Riffenburg[2] found that water in "bad bottles" dissolves glass fairly rapidly and becomes quite appreciably alkaline, and Long[3] showed that alkali decomposes tartar emetic with the precipitation of antimony oxide. With these considerations in mind, a 5 per cent solution of pure tartar emetic in cooled, freshly distilled water was sealed in three types of bottles: (1) a paraffined bottle, (2) a hard glass bottle, and (3) a soft glass bottle, so that all air was excluded. After four months these solutions had undergone no visible change and the optical rotatory power was exactly that of the original solution; the latter is a delicate means of detecting decomposition. The solution in the hard glass bottle was then refluxed for five hours in contact with air, without causing any change in appearance or optical rotation. Another 5 per cent solution remained unchanged after being shaken with air frequently every day for three weeks. In another case a solution which had been treated with CO_2 daily for three weeks, allowed to stand three months in contact with air and then autoclaved for one hour at twenty-six pounds steam pressure, showed no signs of decomposition.

These experiments prove conclusively that solutions of pure tartar emetic in distilled water do not undergo decomposition under the conditions to which such a solution is likely to be exposed, and that the solution remains perfectly clear after sterilization. It is obvious that strong acids and alkalies, and physiologic solutions, such as those containing calcium, must be kept out of the tartar emetic solutions as these cause decomposition.

In order to make these results more general, a sample of pure sodium antimonyl tartrate[4] was subjected to the tests applied to the potassium salt. The calculated value for antimony in sodium antimonyl tartrate ($C_4H_4O_7NaSb.\frac{1}{2}H_2O$) is 37.98 per cent, and that found in our material is 37.91 per cent. The solutions of this substance proved to be just as stable as those of tartar emetic.

*From the Department of Pharmacology and of Tropical Medicine, Harvard Medical School.

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As this work was done entirely with highly purified specimens, attention was directed toward commercial specimens of tartar emetic. Nine samples have been secured from different sources,[5] and none of these dissolves to a clear solution; the amount of insoluble matter varies from a trace to 0.14 per cent. A number of these specimens were recrystallized from water; the purified material in each case dissolved to a perfectly clear solution. The insoluble material was examined in four cases; in addition to large amounts of antimony it was found to contain small amounts of arsenic, iron, and lead. Antimony determinations showed that the recrystallized products contained less antimony than the original, corresponding to the fact that the commercial material contains some antimony oxide not combined with the potassium bitartrate.

TABLE I

SAMPLE	SB CONTENT %	INSOLUBLE MATTER	SB CONTENT AFTER RECRYSTALLIZATION	ANALYSIS OF INSOLUBLE RESIDUE
A	36.95	++++++	——	——
B	36.57	++++	——	——
C	36.85	++++	——	——
D	36.85	+	——	——
E	35.80	++++++++	32.67	Sb, As, Fe, Pb
F	36.73	+++++	35.10	Sb, Pb
G	38.13	+	36.07	Sb, Pb
H	37.97	+	36.15	——
I	——	+	——	Sb, As, Fe, Pb

The arsenic and iron found in the insoluble matter is always considerably less than the total arsenic and iron in the commercial samples, indicating that these impurities are present mainly in soluble compounds. The iron compound remains in the mother liquor under all conditions during recrystallization. To remove the arsenic completely, the solution must be boiled quite vigorously prior to filtration; this decomposes the arsenical, liberating the latter in a soluble form.

In preparing tartar emetic, the usual procedure is to boil antimony oxide with an aqueous solution of potassium bitartrate, and after removing the unchanged antimony oxide by filtration, the potassium antimonyl tartrate crystallizes from the filtrate. Unless great care, or some clarifying agent such as vegetable carbon, is used during the filtration, some of the excess antimony oxide passes through the filter and contaminates the product. The water insoluble material found in commercial specimens is due to faulty manipulation at this point; a perfect filtration at this stage enables one to obtain tartar emetic which dissolves to an absolutely clear solution, whereas a careless filtration permits a little antimony oxide to pass through and the resulting product is not completely soluble in water. The arsenic found in tartar emetic is introduced by use of antimony oxide containing arsenic as an impurity, and the lead is due to the presence of the latter in the potassium bitartrate. One cannot help but wonder whether this insoluble matter has not been noted by the clinicians, and constitutes the precipitate which they mention.

Experiments designed to detect any other differences between commercial and laboratory tartar emetics and to determine whether the insoluble material had any bearing on the stability of the tartar emetic solution were undertaken. These were all negative, and the solutions were just as stable as those prepared from laboratory material.

Since the arsenic in tartar emetic is due to the use of impure antimony oxide, two samples of the commercial C.P. oxide were examined, and the results obtained are shown in Table II.

TABLE II

IMPURITY	SPECIMEN *A*	SPECIMEN *B*
Sulfide	Trace	Considerable
Metals other than antimony	None	Trace of iron, small amount of arsenic
Silica	About 1%	Small amount

Although this qualitative investigation shows nothing which should affect the use of these specimens in the preparation of tartar emetic, it has been found that they react slowly when boiled with potassium acid tartrate, and the potassium antimonyl tartrate, isolated from the reaction mixture after two hours, is low in antimony. This is markedly different from the results secured with freshly purified antimony oxide. In this case, an excellent yield of tartar emetic containing the calculated amount of antimony is obtained.

To prepare tartar emetic four grams of antimony oxide and five grams of potassium bitartrate in fifty c.c. of water are refluxed with stirring for two hours. Vegetable carbon is added and the mixture is filtered hot. The clear filtrate is allowed to cool in the ice box overnight, and then the crystals are filtered, washed and air dried.

TABLE III

ANTIMONY OXIDE	SB IN PRODUCT
Commercial A	20.5
" B	25.9
Purified I	36.2
" II	36.3
Calc. for $C_4H_4O_7KSb:\frac{1}{2}\ H_2O$; Sb 36.2%	

Even after refluxing commercial antimony oxide A with the bitartrate for four hours, the product contained only 32.3 per cent antimony. These experiments indicate clearly that it is desirable to use freshly prepared antimony oxide in this reaction. The reaction in question occurs between solid and liquid phases, and one would expect that the rate of reaction would vary with the nature of the surface of the solid, i.e., the antimony oxide. With this in mind, it is readily seen that the freshly prepared (purified) oxide might react much faster than the old commercial material. To purify the oxide it has been found advantageous to dissolve 100 gr. of oxide in 225 c.c. of hot concentrate hydrochloric acid. The filtered solution is evaporated to

75 c.c. to remove part of the excess hydrochloric acid, and then diluted to 1.5 liters. Part of the antimony chloride hydrolyzes, but to secure a good yield aqueous ammonia is added till the hot solution is only slightly acid to Congo Red. After cooling overnight, the antimony oxide is filtered and washed with hot water till free from chloride; a 95 per cent recovery is secured.

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[5]Four of these specimens were obtained from retail drug stores; four others were secured from wholesale houses and the last one (I) came from a large hospital.

CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

By A. Levinson, M.D., Chicago, Ill.

(*Continued from page* 805.)

Skin Tests

Of the numerous skin tests advocated the following have been found most useful:

Tuberculin tests, of which there are the von Pirquet, Mantaux, Subcutaneous, Moro and Calmette.

Schick test.

Noguchi Luetin test.

Protein sensitization tests.

Tuberculin Tests.—There is no agreement in the literature as to which of the tuberculin tests is preferable. Von Pirquet, Moro, Calmette, subcutaneous and intradermal have all been used. I have come to regard the von Pirquet and the intradermal as the most useful tuberculin tests.

The Von Pirquet Test.—An area of the forearm is cleansed with alcohol and followed by ether. Two drops of undiluted Koch's O.T. are placed on the skin about 4 cm. apart. The skin is then scarified with a Pirquet bore or needle at a point between the drops for control and also over the spots containing the O.T. If the test is positive, redness and induration will appear over the areas containing the tuberculin, the control being unaffected (Fig. 47). The reaction usually appears in 24 hours and lasts three to four days.

The Intradermal Tuberculin Test.—Koch's O.T. is diluted 1 to 1000, 1 to 10,000 and 1 to 100,000, with normal salt solution. Two-tenths of a c.c. of the diluted tuberculin is injected intracutaneously. Infiltration and redness indicates a positive reaction (Fig. 48).

Interpretation.—A positive tuberculin test means nothing more or less than that a tuberculous infection is present somewhere in the body. It does not, however, localize the infection, neither does it tell whether or not the infection is active at the time the test is made. The tuberculin test is of

great importance in children in that it tells whether or not the child has been infected by tubercle bacilli.

SCHICK TEST.—Schick found that when $\frac{1}{50}$ of a minimal lethal dose of diphtheria toxin is injected intradermally, an area of redness and infiltration will appear at the spot of injection if the patient has not at least 0.03

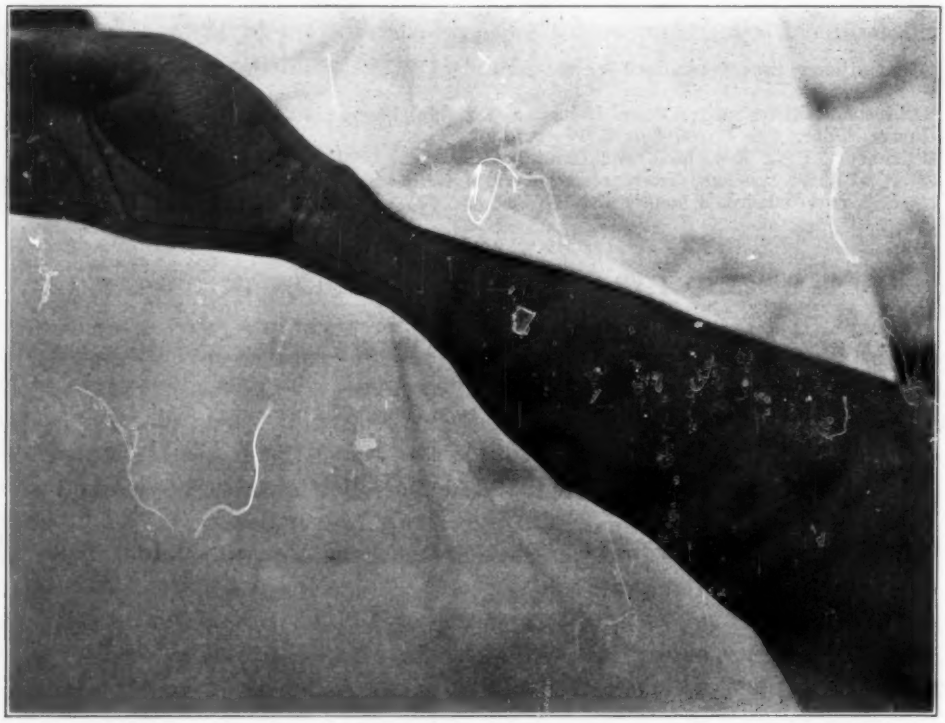

Fig. 47.—Positive von Pirquet reaction.

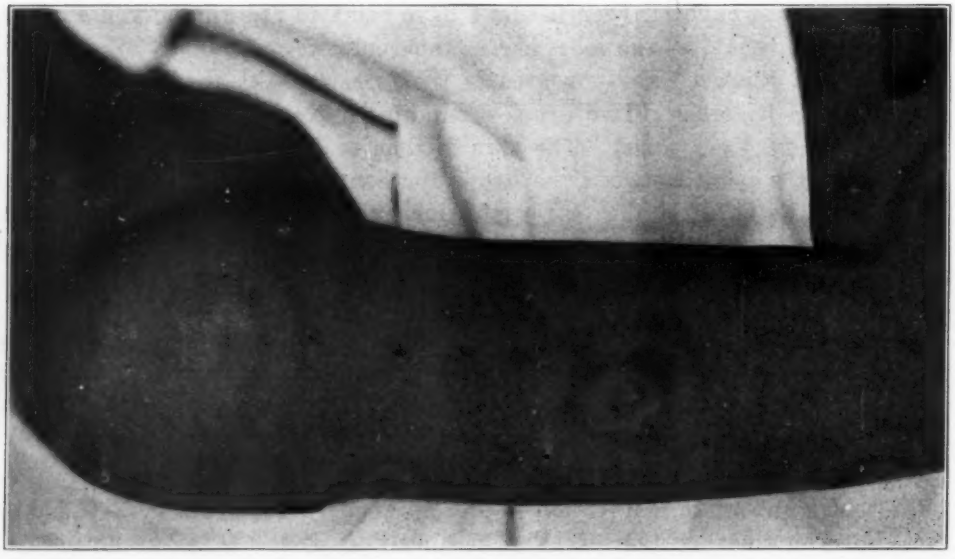

Fig. 48.—Positive intracutaneous tuberculin test.

unit of antitoxin per cm. of blood. In other words, a positive reaction will take place if the patient is susceptible to diphtheria.

Technic.—Two-tenths c.c. of diluted diphtheria toxin (obtained from most health departments and some commercial houses) is injected into the forearm or back by means of a thin, short needle, care being taken that the solution is injected intradermally. The skin at the area of injection shows a whitened appearance with definite indentation if the fluid has been introduced intracutaneously instead of subcutaneously.

If the patient is susceptible to diphtheria, redness and infiltration will occur in 12 to 24 hours. Needle track redness should not be considered a positive reaction.

A positive Schick reaction does not necessarily mean that the disease in question is diphtheria; yet, if the patient showing the positive reaction has been exposed to diphtheria, he should receive the proper amount of antitoxin.

Toxin Antitoxin.—It is advised that children over five years of age who give a positive Schick reaction, should be immunized by toxin antitoxin (diphtheria toxin almost completely neutralized by antitoxin). Three injections are given subcutaneously at 1 week intervals. In children under five years of age, toxin antitoxin is advised even without a preliminary Schick test.

Immunity is supposed to develop from two to six months after the injection of toxin antitoxin and to last five years.

Protein Sensitization.—It has been found that some children develop an urticaria and even an asthmatic attack upon ingestion of certain protein. Other children have an asthmatic attack on coming in contact with certain pollen. A patient's susceptibility to protein may be determined by applying the suspected protein on the skin.

Technic.—The various proteins are obtained on the market in concentrated form. After the forearm has been washed with alcohol and dried with ether, one drop of a tenth normal solution of sodium or potassium hydroxide is applied to the skin by means of a sterile toothpick. A small amount of protein is now rubbed over the area containing the sodium or potassium hydroxide. If the patient is susceptible to the protein, the skin surrounding the tested area will show an urticaria and induration within ten to thirty minutes.

The following are the most important proteins from a pediatric standpoint.

Food Proteins.—

Carrot	Cow's milk	Spinach
Cheese	Oatmeal	Strawberry
Chicken	Orange	Tomato
Egg white	Pea	Veal
Egg yolk	Sweet potato	Wheat
Human milk	Rice	

Pollen Proteins.—

Goldenrod	Juniper	Rye
Ragweed	Oats	

Other Proteins.—

Horse Serum

Smears and Cultures

Among the most important smears used in clinical pediatrics are the following:

1. From the mucous membrane of the mouth for Vincent's organism, and for Odium albicans in thrush.
2. From the throat for Klebs-Löffler bacilli, and for Vincent's organism.
3. From the nose for Klebs-Löffler bacilli.
4. From the conjunctiva for gonococci, diphtheria, and for the Koch-Weeks bacilli.
5. From vulva and vagina for gonococci.
6. From urine for the examination of tubercle bacilli and typhoid bacilli.
7. From stool, for examination of intestinal parasites and their ova.
8. From sputum for tubercle bacilli.
9. From skin lesions, such as in scabies, favus, and ringworm.
10. From various exudates.

The most common cultures taken from children are:

1. From throat and nose for the examination of Klebs-Löffler bacilli, and for meningococci.
2. From blood for various organisms, especially streptococci and typhoid.
3. From urine for typhoid and colon bacilli.
4. From stools, for typhoid bacilli, dysentery bacilli, especially the Flexner type, for gas bacilli, and for streptococci.
5. From milk for diphtheria bacilli and streptococci, especially during epidemics of diphtheria, scarlet fever, and streptococcus sore throat.

Smears from the Mouth.—In all forms of stomatitis and in thrush a smear should be taken from the mouth, in search of the spirillum of Vincent, which may cause fatal infection unless treated early with some arsenic preparations. In making a smear for the Vincent's organism the mucous membrane of the mouth is swabbed with a cotton applicator, and the material is put on a slide. The slide is dried in the flame and stained with carbolfuchsin, although methylene blue may answer the purpose, and examined under the microscope (Fig. 49).

For the detection of the organism in thrush the mucous membrane of the cheek is swabbed and applied on a slide, on which a few drops of 10 per cent NaOH has been placed.

Smears from the Throat and Nose.—The identification of Klebs-Löffler bacilli in a direct smear from the throat, is difficult and unreliable, and should, therefore, be used only as corroborative evidence in making a positive diagnosis, the smear being followed by a culture. The same applies to smears from the nose.

When Vincent's angina is suspected, smears should be made for the presence of spirillum.

In all conjunctival exudates, especially in the newly born, smears should be made from the conjunctival exudate and examined for gonococci. Occasionally the exudate of the conjunctiva is dried up and a thin layer covers

over the pus. In making conjunctival smears, therefore, the applicator should be rubbed firmly over the conjunctiva.

Occasionally one encounters diphtheria of the eye, especially in institutional children. A smear may show the Klebs-Löffler organisms.

VAGINAL SMEARS.—Vaginal smears should be made from all vaginal discharges, and from all female children admitted to a children's hospital to prevent epidemics of vaginitis.

The smear is made on a glass slide by means of a cotton applicator. The slide is dried in the flame and stained with methylene blue and if found suspicious also with Gram stain. Gram negative intracellular organisms should be considered gonococci (Fig. 50).

SMEARS FROM URINE.—Whenever tuberculosis of the genitourinary tract is suspected, the urine, after standing several hours in a refrigerator, should be centrifuged for an hour or two and examined for tubercle bacilli.

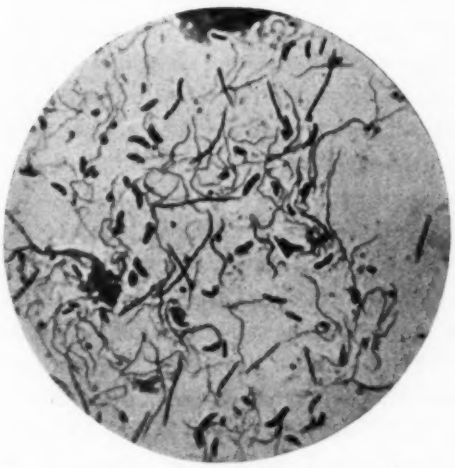

Fig. 49.—Throat smear from case of Vincent's angina.

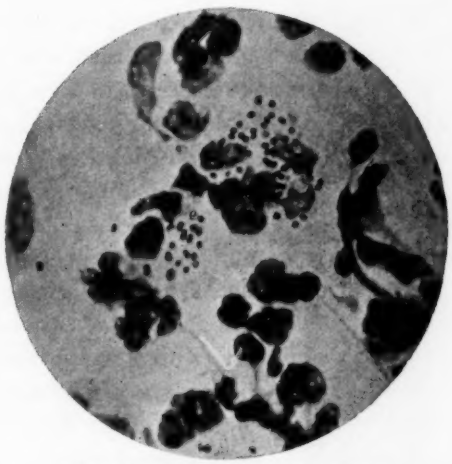

Fig. 50.—Positive vaginal smear.

When typhoid is suspected, the urine should be centrifuged and examined for typhoid bacilli.

SMEARS FROM STOOL.—Smears from stools should be examined under the microscope for blood, for fat, and for parasitic ova. In examining for blood an unstained specimen may be used. In examining for fat, a drop of a saturated solution of sudan III should be put on a slide. Fat appears as bright red droplets scattered through the field. In examining for ova, a saturated solution of sodium chloride should be used (method described in section on stool). In looking for Ameba dysenteriae the stool should be examined as soon after removal from the body as possible, and should be kept warm until put under the microscope, or a special apparatus should be used to keep the stage of the microscope warm.

SMEARS FROM SPUTUM.—Children seldom raise sputum. When desired for examination, sputum may be obtained from children by irritating the pharynx with a tongue depressor. Children seldom suffer from the ulcerative

forms of tuberculosis of the lungs, so that only occasionally does one have to examine sputum from children for tubercle bacilli. Typing of pneumococci in sputum is being done by many workers.

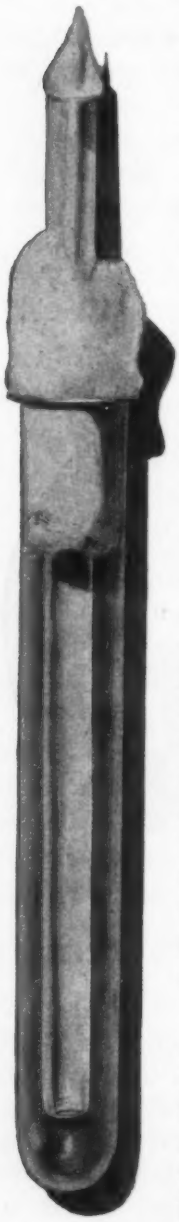

Fig. 51.—Special specimen tube for stool cultures.

In looking for skin fungi, exudate, scales, or hairs of the affected area are placed on the slide on which has been put a few drops of 10 per cent sodium hydroxide. The specimen is examined unstained for large spores, for scabies, and for ringworm.

EXUDATES.—Fluid obtained from the body should be examined on a slide for the number and type of cells, and for organisms. If the exudate is cloudy it is not necessary to centrifuge it. If thin, it should be centrifuged and the sediment used for smears.

In empyema or in abscess of the ears, a bacterial count may be used as an indication of the progress of the case. For clinical purposes the pus is put on a slide and a portion of the smear in which the cells are equally divided is selected and the bacteria in 10 successive microscopic fields, including those fields that contain no bacteria, are counted. The number of bacteria found in these 10 fields is then divided by 10 and the average number per field obtained.

THROAT AND NOSE CULTURES.—Any exudate in the throat and any discharge of the nose should be cultured for Klebs-Löffler bacilli. This is especially true with reference to the nose. Many a case of nasal diphtheria is overlooked because the clinical symptoms are not so acute as in other forms of diphtheria. This subacute and dangerous form of diphtheria could be easily detected by a culture from the nasal discharge. It is a good rule to culture all nasal discharges, especially if they are mucopurulent or bloody. It should be pointed out that a patient whose culture contains organisms suspicious of diphtheria should receive antitoxin immediately, without waiting for a report of the culture.

Technic.—The throat is swabbed by means of a sterile swab (supplied by most Boards of Health), and the swab rubbed on Löffler's blood serum. Care should be taken that the culture medium is not dried out, in which case no growth will take place. The box or test tube containing the medium (supplied by most Boards of Health) is incubated in the incubator for 12 to 18 hours, or in the vest pocket for 18 to 24 hours, and the growth is put on a slide, stained with methylene blue, washed off with 0.3 per cent acetic acid and water and examined under the microscope with the oil immersion lens. If the culture is positive, or even only suspicious, the patient should receive antitoxin without delay.

Occasionally the throat culture is positive, and yet the child does not

suffer from an active diphtheria, and does not need any antitoxin. This is the case with diphtheria carriers. Still, it is best to follow the rule that each patient with a positive culture should receive antitoxin.

To discover meningococci carriers the postnasal spaces are swabbed by means of a bent applicator, blood agar being used as a culture medium.

A blood culture should be used at the height of the disease for the determination of the offending organism (technic described in section on blood).

CULTURES FROM URINE.—From urine, cultures should be made only from specimen obtained by catheterization, otherwise the culture will be contaminated to begin with. Typhoid and colon bacilli constitute the most important organisms looked for in cultures from urine. Typhoid bacilli are examined for the purpose of diagnosis and colon bacilli for diagnosis and treatment; the latter when vaccine is contemplated for the treatment of pyelitis.

STOOLS.—In making cultures from stools it is best not to use the exposed surface of the stool but to remove the upper layer by a clean wood applicator. A small portion of the stool is transferred to the culture medium. If contamination by all other bacteria than the one looked for is to be completely avoided, a sterile, long glass rod or tube with the edges of the tube well rounded so as not to injure the rectum is introduced high up into the rectum, and a specimen obtained. This is transferred into the culture medium or into a sterile tube and closed with cotton (Fig. 51).

TABLE XV

TABLE SHOWING STAINING REACTION OF VARIOUS COMMON BACTERIA TO GRAM STAIN

POSITIVE	NEGATIVE
Cocci-Staphylococcus pyogenes aureus	Meningococcus
Staphylococcus pyogenes citreus	Gonococcus
Staphylococcus pyogenes albus	Micrococcus catarrhalis
Streptococcus hemolyticus	
Streptococcus mucosus	
Streptococcus viridans	
Micrococcus tetragenus	
Pneumococcus (Fraenkel)	
Bacilli	Pneumobacillus
Diphtheria (Klebs-Löffler)	(Bacillus mucosus capsulatus of Friedlander)
Pseudodiphtheria (Hoffmann)	Typhoid
Leprae	Paratyphoid } Typhoid colon group
Tuberculosis	Colon }
Anthrax	Dystentery }
Tetanus	Influenza
Aërogenes capsulatus (Welchii)	Spirillus fusiformis of Vincent
Oidium albicans	Malignant Edema
	Pyocyaneus
	Cholera
	Koch-Weeks (Pink-eye)
	Morax-Axenfeld
	Bordet-Gengou (Pertussis)

Routine Stains

A. Methylene Blue (Loeffler's Alkaline).
   1. Fix—with heat.
   2. Stain 30 seconds.
   3. Wash quickly, dry on filter paper.

B. Gram Stain.
   1. Fix—with heat.
   2. Stain with carbol gentian violet—30 seconds.
   3. Add Gram's iodine—1 minute.
   4. Wash with absolute alcohol until smear is faint pink.
   5. Wash with H_2O; let some water remain on slide, and
   6. Add Safranin (5-6 drops for 15 seconds).
   7. Dry with filter paper.

C. Stain for tubercle bacilli.
   1. Dry smear in air or incubator and heat.
   2. Stain with carbol fuchsin (Ziehl-Neelsen)—let it simmer on flamed tripod 3 minutes or more.
   3. Add acid alcohol until smear is faint pink.
   4. Wash with water—let water remain on slide
   5. Add methylene blue—4-5 drops for 30 seconds.
   6. Dry with filter paper.

D. Capsule Stain (Welch) (milk cultures used).
   1. Dry in air.
   2. Fix and add glacial acetic-acid. Let stand 10 seconds.
   3. Dry with filter paper.
   4. Add carbol gentian violet—leave it on for 30 seconds.
   5. Wash with 2 per cent solution of NaCl.
   6. Dry with filter paper.

E. Wright Stain.
   1. Stain 3 to 5 minutes, according to strength of stain. Count the drops.
   2. Add an equal number of drops of distilled H_2O until metallic film appears. Let stand 2 minutes.
   3. Wash with tap water.
   4. Dry with filter paper.

THE IMPORTANCE OF THE ADRENAL GLANDS IN THE ACTION OF CERTAIN ALKALOIDS*

III. Physcstigmine on the Blood Picture

By Charles W. Edmunds, M.D., and Putnam C. Lloyd, A.B.,
Ann Arbor, Michigan

IT has been shown in two earlier papers[1, 2] that the adrenal glands may play a distinct rôle in producing some of the changes in the body which follow the injection of certain alkaloids. Those alkaloids which have been studied are pilocarpine, physostigmine and strychnine, all of which have been shown to stimulate the adrenals. The first named, it is true, stimulates the glands only to a very slight degree while the other two have been shown to increase the amount of epinephrine in the blood even up to fifteen or twenty times the normal. (Stewart and Rogoff.)

In the first paper named it was shown that the increased amount of epinephrine secreted could offset the direct action of pilocarpine upon certain nonstriated muscular structures, viz., the uterus and the intestines. In the second paper, it was shown that the epinephrine secreted under the influence of strychnine was present in sufficient amount to produce characteristic changes in the number of white blood cells.

The investigation was continued by an examination of the effects of physostigmine, studying especially the changes in the blood counts following the injection of the alkaloid. In the work, the blood changes were studied as in the case of strychnine reported in the earlier paper,[2] taking the counts of both red and white cells and also blood spreads from which differential counts were made. Dogs were used as before, and all were given chloretone as an anesthetic, the same being supplemented by paraldehyde by mouth in case it was necessary. The blood, as a rule, was obtained by a prick on the nose but in certain cases it had to be obtained from other localities, such as the neck vessels, though this did not essentially modify the course of the curve, as was discussed in the earlier paper. The physostigmine was given subcutaneously in doses of 0.5 mg. per kilo. These doses were later increased to 0.7 mg. per kilo as will be noted.

EXPERIMENTAL WORK

A number of dogs served as controls, and a typical curve of the blood changes produced by physostigmine is shown in Chart 1. Here again, as in the case of strychnine, the leucocyte count and also the differential count of the white cells show changes which are typical of epinephrine, the in-

*From the Pharmacological Laboratory of the University of Michigan.
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crease in polynuclear leucocytes being mainly responsible for the leucocytosis, although early the lymphocytes are also slightly increased. For comparison, a curve showing a typical epinephrine effect is given in Chart 2. The leucocytosis produced by physostigmine lasts for two hours, although the high point is usually reached in an hour and a half after the alkaloid is given. With 0.5 mg. doses of physostigmine per kilo of body weight, no change in the number of red blood cells is seen.

After the first two or three dogs had been studied, the dose of physostig-

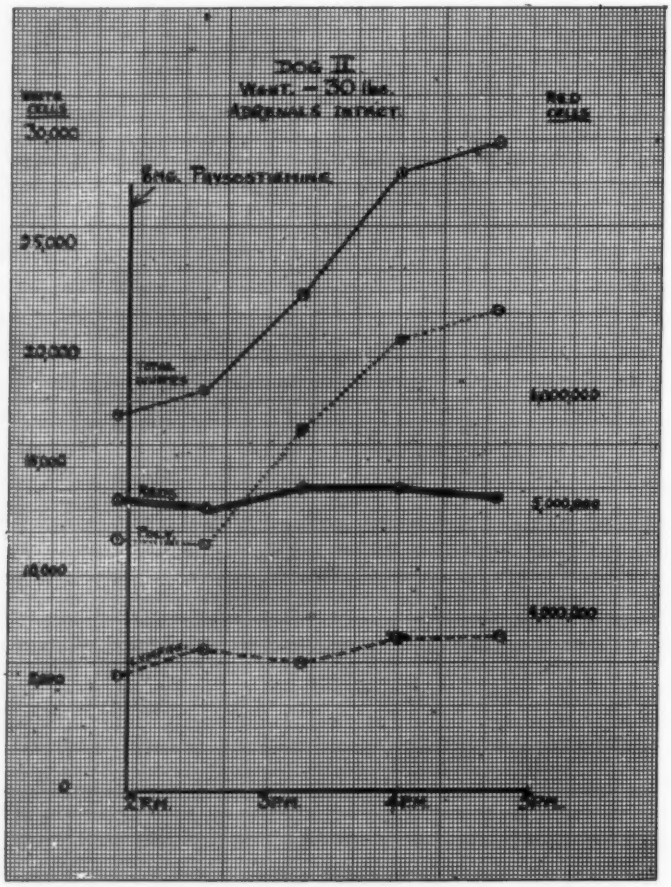

Chart 1.—Blood count in dog following physostigmine in dose of 0.5 mg. per kilo body weight. The adrenal glands were intact. No change in red cell count. Marked increase in polynuclears. Compare with Charts 2 and 3.

mine was increased to 0.7 mg. per kilo of body weight without any difference being noted from the changes described above in so far as the white cells are concerned, but now the red blood cells were found to be increased, as is shown in the curve given in Chart 3. As a result of this finding, in all subsequent dogs the larger dose of 0.7 mg. per kilo was given, and it was found to be very satisfactory to bring out the increase in red cells in addition to the changes in the whites.

In comparing the results, it is very interesting to note the similarity in

reaction which exists between physostigmine and epinephrine in their relation to the blood changes. The type of curves is the same, and in addition both alkaloids act upon the white cells only when given in small doses, while with larger amounts they increase the number of red cells also (Charts 2 and 3).

In a second series of dogs, all procedures were carried out as in the first group, except that before the physostigmine was given, both adrenals were removed. The abdomen was then closed and the animal allowed to remain quiet for a time to permit readjustment and stabilization of the blood distribution. Physostigmine was then injected as before and a typical curve obtained from the blood counts is shown in Chart 4. The result is the same as was found in the case of strychnine, at least in so far as the white cells

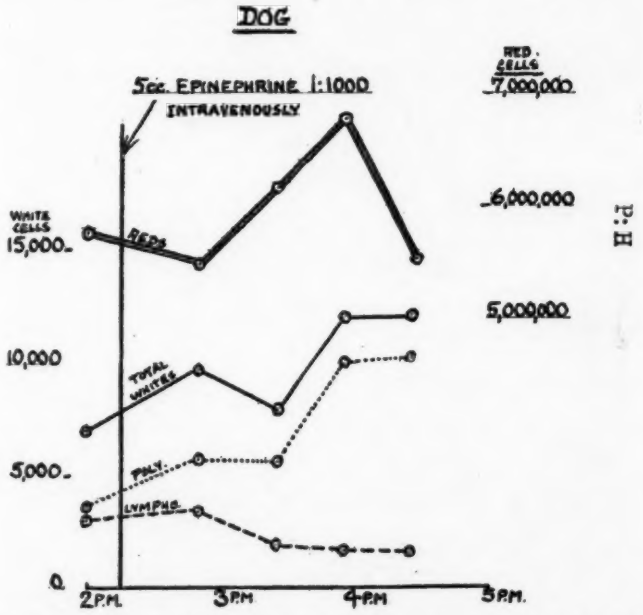

Chart 2.—Blood count from dog following epinephrine. Compare the white blood count with Chart 1.

are concerned. The typical epinephrine-like curve is lost and in its place a decrease in the number of white cells is found in which both varieties of cells participate. The curve of the red cells, however, is not changed in the least, indicating that the increase in these cells was not due to the activity of the adrenal glands but to the action of the physostigmine itself.

The conclusion to be drawn in the case of physostigmine is the same as with strychnine, viz., that the increase in the number of leucocytes and the change in their relative proportions are due to the epinephrine which is poured out by the adrenals under the stimulating effects of the physostigmine. Only one animal was an exception to the above rule. In this dog, both adrenals were removed and the physostigmine administered, but when the blood counts were made it was found that the plotted curve was typical for an epinephrine action. The most probable explanation is that the animal

possessed accessory adrenal glands which reacted to the physostigmine after its normally placed glands had been removed.

Other interesting relations were found in this connection which have a bearing upon the physiology of these glands. It was shown above that the surgical removal of the adrenals prevented the leucocytosis due to physostigmine. Therefore, any influence which will remove these glands function-

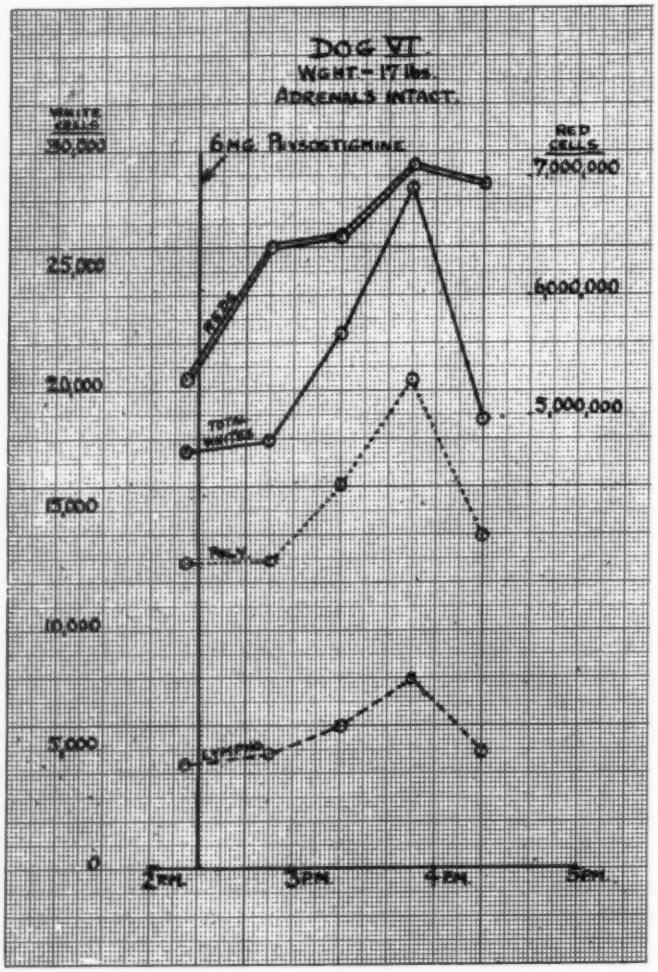

Chart 3.—Blood count in dog with adrenal glands intact following physostigmine 0.7 mg. per kilo. Note increase in red cell count. Compare with Charts 1 and 2.

ally should have the same effect. Such has been found to be the case. Edmunds and Stone[3] showed that ergotoxin will prevent the epinephrine increase in number of leucocytes, and the same was found true for the physostigmine leucocytosis as is shown in Chart 5, where there is a complete reversal of the polynuclear curve. It will be seen here also that the ergotoxin had no effect upon the increase in red blood cells (although it does on the true epinephrine polycythemia), thus demonstrating an essential difference

between the action of physostigmine and of epinephrine upon the number of the red cells.

The action of atropine upon the physostigmine effects was also studied on account of its well-known action in antagonizing that alkaloid elsewhere in the body. It has been shown by Stewart and Rogoff[4] that atropine has practically no action upon the adrenal glands so that it could be employed

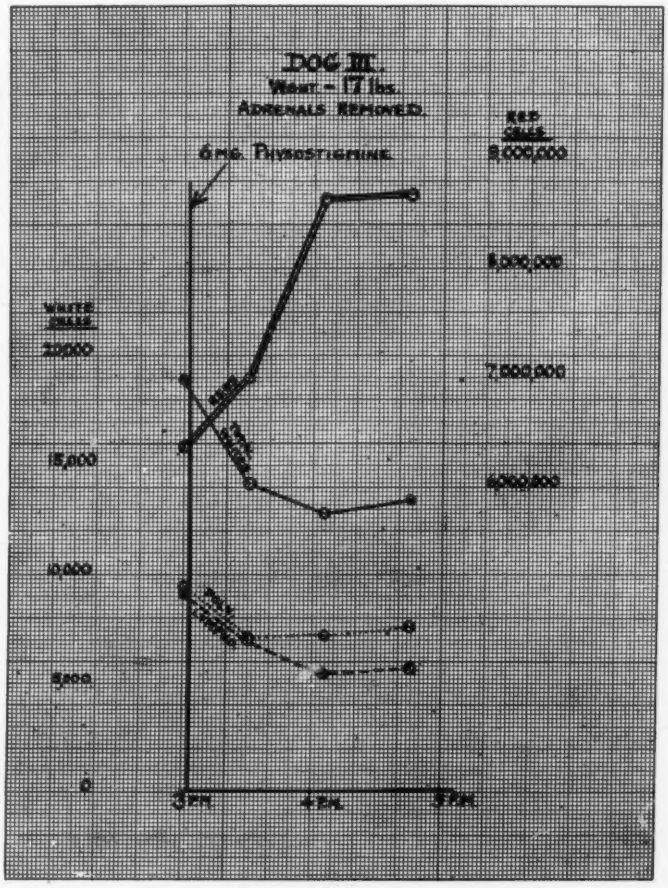

Chart 4.—Dog with adrenal glands removed. Physostigmine 0.7 mg. per kilo. Red cell count is not changed by the removal of the adrenals. Increase in number of leucocytes is absent.

in this connection without any complicating factors coming in. The results were very interesting. With small doses of atropine (3 mg.) opposed by 8 mg. of physostigmine in an 8 kilo dog, the antagonism was partial, the leucocyte curve being kept near the normal height for over an hour. The antagonism was indicated also by the vagus which, following the atropine administration, was found to be inactive but which produced slowing of the heart soon after the physostigmine was injected. The atropine exerted no influence over the red cell increase produced by the physostigmine. In another dog weighing 7 kg., atropine was given in larger amounts (60 mg.)

so as to insure complete antagonism to the physostigmine which was given in a 7 mg. dose. The vagus was examined frequently and was inactive during the entire period of observation. The results are shown in Chart 6, which is a duplicate in all essentials of that which was obtained when the adrenals were removed by surgical means. It is evident that we have here, as in the case of ergotoxin, a complete functional removal of the glands. Also, as with ergotoxin, atropine exerted no influence upon the red cell increase which was brought about by the administration of physostigmine.

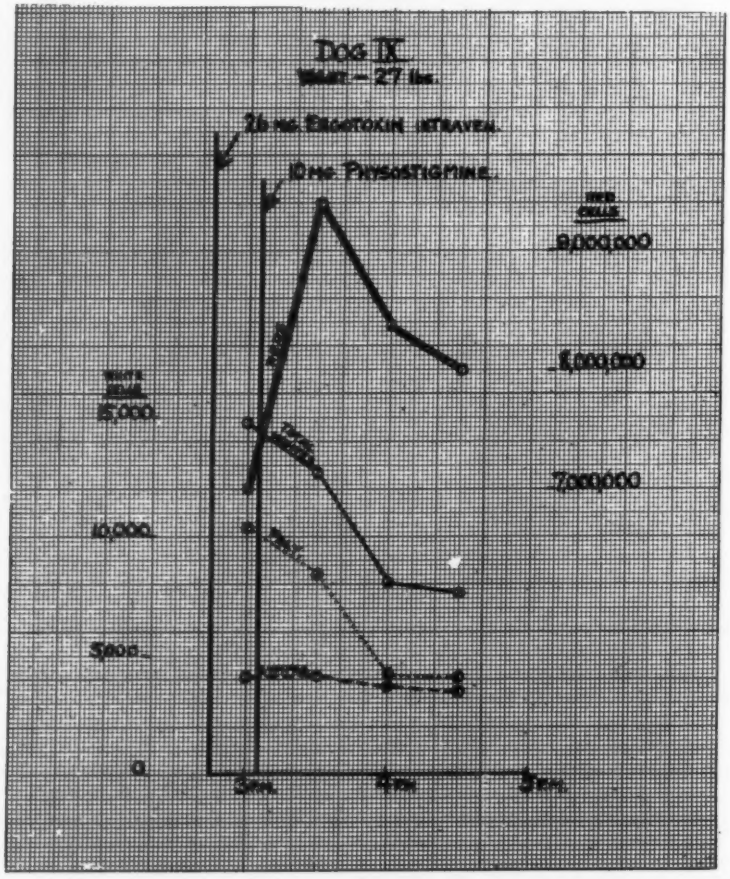

Chart. 5.—Dog with intact adrenal glands. Effect of physostigmine following a sufficient dose of ergotoxin to paralyze the vasomotor nerves.

The effect of curara was also examined on account of the complete antagonism which it shows elsewhere toward physostigmine. As was to be expected, large doses of curara were necessary to maintain a complete paralysis during the entire experiment, 200 mg. being given, a small amount by intravenous injection, the remainder subcutaneously. The result was the same as with atropine, a complete nullification of the physostigmine leucocytosis with no inhibition of the red cell increase. Curara, then, like atropine, had produced a functional removal of the adrenal glands.

DISCUSSION

In any consideration of the action of physostigmine upon the number of blood cells, it is evident that there are two factors concerned: first, the action of the alkaloid itself to increase the number of red blood cells; and second, the action of the epinephrine produced by the adrenals under the stimulating influence of the physostigmine, but present in the blood in sufficient amount to affect the number of white cells only. The two factors must therefore be treated separately, and for the sake of convenience, the

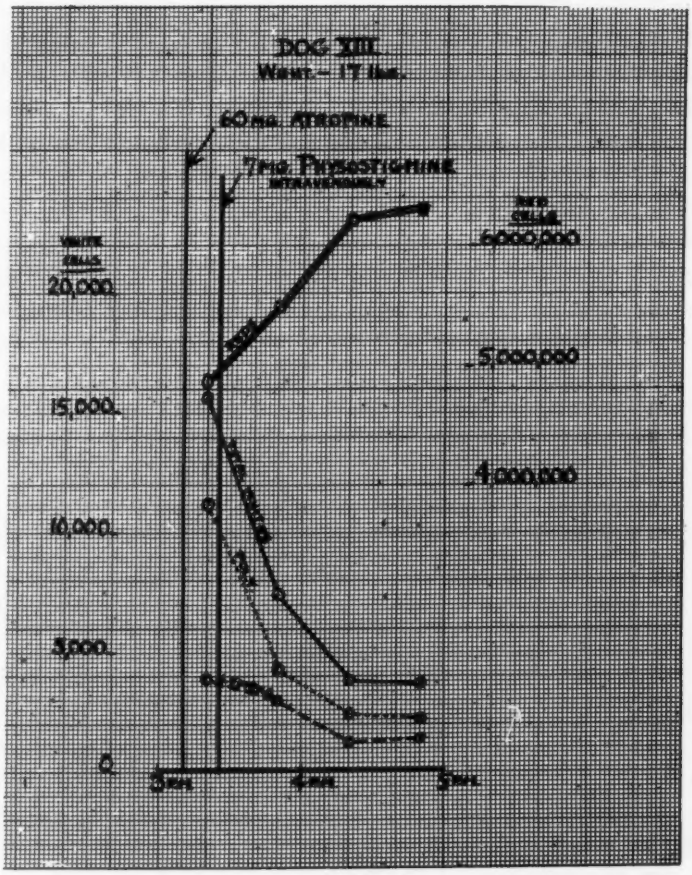

Chart 6.—Dog with intact adrenal glands. Effect of physostigmine following large dose of atropine. Compare with Chart 4.

epinephrine effect upon the white cells will be discussed first. The views upon this aspect of the work must, of course, be brought into harmony with the results obtained by Edmunds and Stone[3] in their study upon the effects of epinephrine upon the blood count. As a matter of fact, they lend weight to the views there expressed as the physostigmine results agree with those obtained by the administration of epinephrine itself.

So far as the physostigmine leucocytosis is concerned, therefore, it is an epinephrine effect and subject to the rules for epinephrine, and produced

probably by an action upon the blood-forming organs as presented in the paper by Edmunds and Stone.

In the case of the increase in the number of red cells following the injection of physostigmine, the problem is perhaps more complex. It is not the same as the polycythemia following epinephrine as it is not affected by the previous injection of ergotoxin. It is of peripheral origin, as is shown by the fact that it is obtained in an eviscerated animal and also after the administration of ergotoxin, atropine and curara, drugs which act on the periphery. It does not seem to be a phenomenon of altered distribution as essentially identical counts are obtained from the capillaries of the nose, from the arteries of the neck, and from the splenic vein.

There are two other possible explanations which it is important to consider. In the first place as to whether the increase is the result of concentration of the blood, or whether it is the result of stimulation of the blood-forming organs especially of the bone marrow. Indeed it is possible that both actions may be involved.

In favor of an action upon the bone marrow is the fact that certain cells, such as normoblasts and young forms of leucocytes which are found normally in the circulating blood in only extremely small numbers, are found at the acme of the drug's action in increased percentages. It will be remembered also that Walterhofer,[5] after injecting animals repeatedly with epinephrine, found definite changes in the bone marrow. If further work, now in progress, should show that it was the result of a direct effect upon the bone marrow, it is probable that it is not a true stimulation to increase production of cells but to a stimulation causing an outpouring of cells already formed in the marrow but which, for some reason, possibly their immaturity, have not been passed out into the general circulation.

There are other reasons for taking this view. The rapidity of action of the physostigmine is against the possibility of a new formation of cells being involved. Then, so far as we have been able to see at present, it is not possible to superimpose a physostigmine polycythemia on an epinephrine polycythemia. Either alkaloid will have its effect in producing an increase of one or two million red cells, but if the other alkaloid is now given practically no further increase takes place. Probably only a certain number of cells are in a proper stage to be released from the bone marrow, and these are sent out under the influence of the first stimulus and no more can be delivered by the second.

In favor of the view that the increase in red cells is the result of a concentration of the blood is the fact that, in quite a number of the experiments, there was a certain degree of parallelism in the curves denoting the number of red cells and that indicating the number of leucocytes. The parallelism in no case was complete, but was very suggestive in many cases. On the other hand in many of the experiments the curves denoting the red and the white cells go in opposite directions, showing that concentration of the blood could not be entirely responsible for the changes. Also the part played by the presence or absence of the adrenal glands is against the con-

centration theory. Further experiments are under way in which it is hoped that it will be possible to show which one of these factors is responsible for the changes in the number of red blood cells, or whether both are involved in the process.

CONCLUSION

Physostigmine, of itself, produces no increase in the number of white blood cells in the circulating blood of the normal dog. The leucocytosis, which follows its injection, is due to the epinephrine which is present in increased amounts in the blood due to the stimulation of the adrenal glands by the physostigmine. The leucocytosis is absent if the adrenals are removed before the physostigmine is given. It is, therefore, an epinephrine effect and is not due to physostigmine itself. It is entirely analogous to the leucocytosis following the injection of strychnine. It is not necessary that the adrenals be removed by surgical means in order to prevent the leucocytosis. Since physostigmine (and strychnine) act upon the spinal cord to stimulate the glands, drugs which break the connection between cord and glands will functionally isolate the glands and thus prevent their stimulation with resulting leucocytosis. This functional removal of the gland is accomplished by ergotoxin, atropine and curara.

Physostigmine given in small doses (0.5 mg. per kilo) to dogs causes no characteristic change in the number of red blood cells, while with slightly larger amounts (0.7 mg. per kilo) it markedly increases their number in the circulating blood. This increase seems not to be due to differences in distribution of the cells but to an action upon the bone marrow, although changes in the concentration of the blood may play a part. Further experiments are being carried on in an effort to ascertain the relative importance of these two factors, in case both are involved.

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COMPARATIVE STUDIES IN THE CHEMISTRY OF BLOOD AND CEREBROSPINAL FLUID*

II. Calcium, Magnesium, and Phosphorus

By Nieves G. Barrio, M.D.,† Rochester, Minn.

A DETAILED study of the organic constituents of the cerebrospinal fluid in a series of patients known, or suspected, to have syphilis, with special reference to the relation between the concentration of these constituents in the spinal fluid and the blood has recently been reported by Egerer-Seham and Nixon. A similar study of the inorganic constituents of the cerebrospinal fluid is reported here, and data on the calcium, magnesium, and phosphorus content of this fluid are given in Tables I, II, and III. The sodium and potassium content of the fluid was determined at the same time, and will be reported by McVicar and Ross in a separate communication from this laboratory.

TECHNIC OF EXPERIMENTS

Calcium was determined by the method of Kramer and Tisdall. The method of Bell and Doisy was used for inorganic phosphorus. Magnesium was determined by precipitation as ammonium magnesium phosphate, according to the directions of Kramer and Tisdall. The precipitate was then collected and washed by centrifugalization, first with 2 per cent ammonia and then with 95 per cent alcohol, made alkaline with ammonia. The alcohol was removed by evaporation on a water-bath; the precipitate was then dissolved in 0.01 normal hydrochloric acid and the excess acid titrated with 0.01 normal sodium hydroxide, using cochineal as an indicator. This method when applied to blood is accurate to about 0.4 mg. for each 100 c.c. A further review of analyses for magnesium, using the method of Hammett and Adams, will be reported later.

The spinal fluid was obtained from patients, who for the most part were undergoing treatment for cerebrospinal syphilis. Egerer-Scham and Nixon did not find marked changes from normal in the organic constituents of the fluid in such patients. The content of calcium, magnesium, and phosphorus in the blood serum of the reported cases is within the normal ranges reported by Myers, and the spinal fluid findings may be accepted as representing approximately the normal quantities of these elements.

*This investigation under the direction of Dr. Leonard G. Rowntree, Division of Medicine, is a continuation of work begun under a grant from the Interdepartmental Social Hygiene Board.

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†Special Student in Medicine, The Mayo Foundation.

SUMMARY

The calcium content of the spinal fluid varied between 4.6 and 6.1 mg. to each 100 c.c. or, on the average, approximately 50 per cent of the concentration of calcium in the blood serum.

The inorganic phosphorus content of the spinal fluid varied between 1.5 and 2.7 mg. to each 100 c.c., or, on the average, approximately 50 per cent of the concentration of phosphorus in the blood serum.

TABLE I

CALCIUM CONTENT OF SERUM AND CEREBROSPINAL FLUID

DIAGNOSIS	CALCIUM, MG. FOR EACH 100 C.C. OF SERUM	CALCIUM, MG. FOR EACH 200 C.C. OF SPINAL FLUID	RATIO OF SPINAL FLUID TO BLOOD, PER CENT
Syphilis	9.6	5.0	52
Syphilis	10.0	4.6	46
Syphilis	11.0	5.3	48
Syphilis	12.2	6.1	50
Syphilis	11.0	4.9	45
Syphilis	10.5	5.1	48
Syphilis	10.2	4.9	48
Syphilis	10.4	4.7	45
Syphilis	10.3	5.1	50
Syphilis	10.5	4.9	47
Syphilis	10.0	5.7	57
Aortitis	10.2	5.4	53
Multiple sclerosis	9.6	4.6	48
Uremia	10.4	5.3	51

TABLE II

MAGNESIUM CONTENT OF SERUM AND CEREBROSPINAL FLUID

DIAGNOSIS	MAGNESIUM, MG. FOR EACH 100 C.C. OF SERUM	MAGNESIUM, MG. FOR EACH 100 C.C. OF SPINAL FLUID	RATIO OF SPINAL FLUID TO BLOOD, PER CENT
Syphilis	1.8	2.1	116
Syphilis	2.4	2.9	121
Syphilis	3.1	3.5	113
Syphilis	5.4	6.1	112
Syphilis	2.6	3.0	115
Syphilis	1.7	1.9	112
Syphilis	1.5	1.9	126
Syphilis	1.0	1.9	190
Tabes	1.1	1.4	127
Aortitis	2.5	3.1	124

TABLE III

PHOSPHORUS CONTENT OF SERUM AND CEREBROSPINAL FLUID

DIAGNOSIS	PHOSPHORUS, MG. FOR EACH 100 C.C. OF SERUM	PHOSPHORUS, MG. FOR EACH 100 C.C. OF SPINAL FLUID	RATIO OF SPINAL FLUID TO BLOOD, PER CENT
Syphilis	3.6	1.7	47
Syphilis	4.6	1.8	39
Syphilis	3.9	1.9	48
Syphilis	2.9	1.6	55
Syphilis	2.9	1.6	53
Syphilis	2.8	1.5	52
Syphilis	3.7	1.6	45
Syphilis	3.5	1.5	43
Syphilis	3.9	2.1	54
Tabes	5.0	2.7	54

The magnesium content of the spinal fluid varied between 1.4 and 6.1 mg. to each 100 c.c. The concentration on the average was 125 per cent of that in the blood serum.

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A WARNING AGAINST THE USE OF ARSPHENAMIN IN THE TREATMENT OF SYPHILIS IN A HEMOPHILIC*

By Jacob Rosenbloom, M.D., Ph.D., Pittsburgh, Pa.

A RECENT experience has taught me the great danger in the use of arsphenamin in hemophilia. Mr. R. T., colored, consulted me for continual bleeding from a tooth-socket following a tooth extraction. This bleeding had continued for two weeks. His hemoglobin was 40 per cent (Dare), red corpuscles 2,300,000, and Wassermann ++++ together with a history of an initial lesion 15 years previous. Transfusion was done.

As the bleeding did not stop following the use of horse-serum and transfusion, an injection of 0.6 grams of arsphenamin was given. Within one hour following the injection, all of his gums commenced bleeding at an alarming rate, the hemoglobin falling to 30 per cent. The bleeding was controlled by the use of adrenalin compresses and another transfusion. In spite of two more transfusions, the use of coagulose, horse-serum, emetin, etc., he continued to bleed, and two weeks following the injection of arsphenamin he died from anaemia due to the hemorrhages.

Oliver and Yamada[1] have shown that if an animal is killed by the injection of a large dose of disodium arsphenamin and the blood is drawn soon after death from the vena cava, it will be found that the red cells have been agglutinated and that the plasma no longer coagulates.

Flandin and Tzanck[2] also showed that arsphenamin prevents the coagulation of blood under such conditions, and concluded that the lack of coagulation was due to the action of the arsphenamin on thrombin or its precursors.

Oliver and Douglas,[3] lately made an experimental study of the factors responsible for the effect of large doses of arsphenamin on the coagulability of the blood. They found that arsphenamin had no marked action on thrombin or its precursors, and that it produced incoagulability of the blood both *in vitro* and *vivo*. They think its action is chiefly on the fibrinogen (globulins) rendering it incoagulable to heat or thrombin.

From the experience related and on the basis of the experimental findings mentioned, I feel that the use of arsphenamin in hemophilia is contraindicated.

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LABORATORY METHODS

A GRAPHIC METHOD OF REPORTING THE WASSERMANN REACTION WITH A NOTE UPON ITS CLINICAL VALUE*

By Robert A. Kilduffe, A.B., A.M., M.D., Los Angeles, Calif.

UNFORTUNATELY for the complete control and efficient treatment of lues, it is, not infrequently, a matter of more or less difficulty to impress upon a certain proportion of cases the vital necessity for prolonged observation. To some extent, as has been pointed out in a previous communication,[1] this is due, perhaps, to an unwarranted reliance placed upon the results of a single Wassermann test, an error more likely to be committed by the patient than by the clinician but one which, all too frequently, is made the basis for premature cessation of treatment with subsequent disastrous results.

It is, of course, as has been noted in a previous report,[2] an impossibility to arbitrarily assign to the Wassermann test a predominant importance in any particular phase of syphilis; it can be maintained, however, that as a control of the efficiency of treatment, and as the most constant single symptom of the disease, it is of paramount importance though, as is well understood, conclusions based upon the results of a single serologic examination must be drawn with extreme care.

In spite of these facts, however, it is a relatively frequent occurrence for an otherwise intelligent patient, when confronted with a diagnosis of syphilis, to inquire as to the time and amount of treatment which will be required to eradicate the disease—a query more easily propounded than satisfactorily answered. It is, nevertheless, a question to which some answer must be made and the reply is necessarily to the effect that this must be largely determined by the results of treatment upon the serologic examinations. This, of course, is the only reply which can be made as the Wassermann test is the most delicate gauge of the effects of treatment as yet available.

An unfortunate concomitant, however, is the fact that a large proportion of Wassermann tests are reported simply in terms of "positive" or "negative," or, at the best, in very roughly quantitative terms of plus-four to plus-one.

It is easily possible, therefore, that a patient with an old neglected, or obstinate infection presenting a plus-four reaction upon the initial test may receive a certain amount of treatment and still, when again tested show a four-plus reaction.

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To the clinician this is readily understandable but to the patient it may be a great and inexplicable disappointment, as he will naturally expect—unless specifically forewarned—some definite and tangible result from the treatment which he has received, whereas, on the contrary, no effect is apparent. At times this may result in a loss of confidence in his physician or the method of treatment, and he may drift from physician to physician or, what is worse, from quack to quack.

For this, and many other reasons, out of the extraordinarily voluminous literature which has accumulated concerning the Wassermann test, one salient point of agreement emerges in which both clinician and serologist are in accord; that necessity for a strictly quantitative method of determining the *amount* of reagin present in the blood at the time of testing.

Lack of uniform adoption of a quantitative test has been very largely due to the nature of the methods heretofore proposed and to the factors of time, labor, and expense involved, but it is practically certain that the Wassermann test of the future, for reasons which are obvious, will and must be a quantitative test.

In my experience the most acceptable technic yet devised is that proposed by Kolmer.[3] In this method the quantitative factor depends upon the testing of the serum in varying amounts of 0.1, 0.05, 0.025, 0.005, and 0.0025 c.c.

The readings are made upon the degree of dilution in which the serum reacts, using a xxxx, xxx, xx, x, and 0 scale and are recorded as indicated below. As the important thing is not the amount of fixation but the *amount of serum* in which fixation is obtained in *any* degree, the reaction is strictly quantitative to an extraordinary degree. The simplest method of reporting the results is to set down the degree of fixation obtained in each serum dilution as a row of "fixation figures" somewhat similar to the method of reporting a colloidal gold reaction, as, for example, 44430, indicating that the reaction was plus four with 0.1 c.c., 0.05 c.c., and 0.025 c.c. plus three with 0.005 c.c., and negative with 0.0025 c.c. of serum.

The degree of positiveness of a serum is determined by the *smallest amount of serum capable of giving fixation,* whatever the degree, fixation in the first four or all five tubes being classed as very strongly positive, fixation in the first three tubes as strongly positive, fixation in the first two dilutions as moderately positive, fixation in the first tube—or 0.1 c.c. of serum only—as weakly positive and negative being, of course, the absence of fixation in any tube of the series.

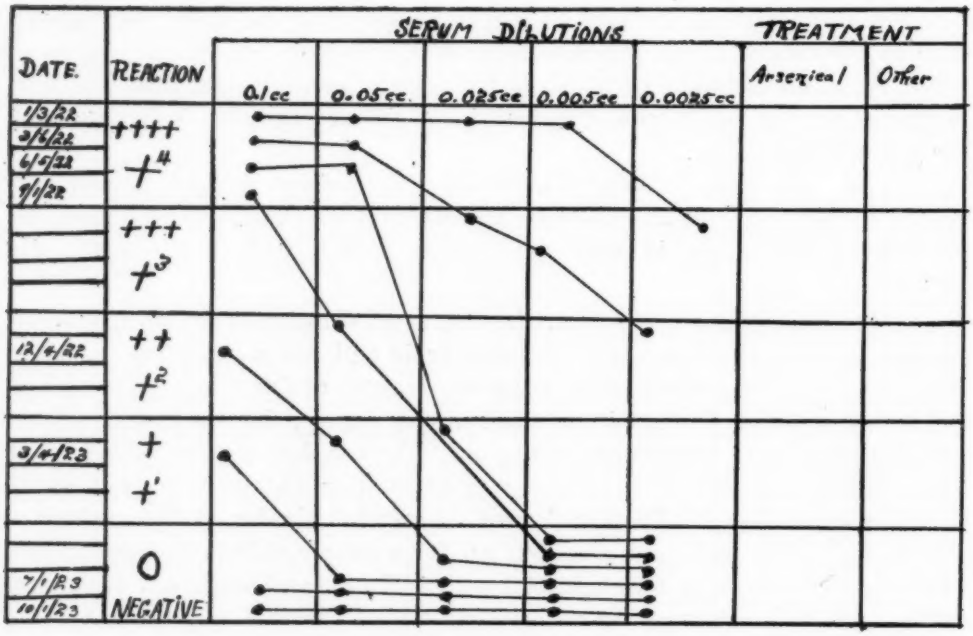
While this is a strictly quantitative and very delicate expression of the positiveness of the reading, this may be even still further refined in that a serum giving a reading of 44444 is obviously a stronger reacting serum and contains more reagin than one which gives a reading of 44322 even though both will be noted as very strongly positive.

The specificity, delicacy, and reliability of this technic have been discussed elsewhere.[4] The purpose of the present communication is to call attention to the ease with which reports by this method may be graphed so

as to vividly represent effects of treatment impossible or difficult to show by a qualitative test.

The appended chart may, if desired, be elaborated to cover a long period of time and to show details of treatment or such other data as may be desirable. It is practically self-explanatory. The ordinates represent the degree of serum dilution tested and the abscissae the reactions obtained, the reaction curves being plotted as shown. The resulting chart shows at a glance the strength of the reaction and forms a ready and graphic method of recording and presenting the result of treatment.

In the case represented the results of the first three serologic examinations, if made by qualitative methods such as are widely used, would almost



certainly have been very nearly alike, probably all plus-four. As graphed when performed by the Kolmer modification, even to the patient it will be evident that, although all three reactions are classed as very strongly positive, a very evident and demonstrable serologic improvement has occurred.

Such a form of report, therefore, presents to the patient visual evidence of serologic gain as a result of treatment, encourages him as to the results to be looked forward to, and is of great assistance as a means of securing his faithful cooperation.

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SOME NEW AND IMPROVED LABORATORY APPARATUS*

By H. C. Sweany, M.D., Chicago, Ill.

WHILE in the construction, organization, and conduct of a large laboratory there are sundry pieces of apparatus that one is called upon to originate to suit a great variety of different purposes, most of these are simple to devise by anyone mechanically inclined, and do not merit description in a publication. Such devices as rat-, mouse-, and rabbit-holders for special experiments, test-tube racks, pipette holders, etc., are a few examples.

There are certain devices, however, that we have been called upon to construct that have been beneficial to us and that may serve a valuable purpose

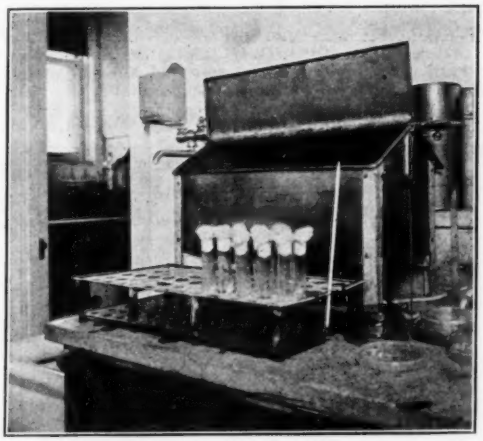

Fig. 1.—Showing the water-bath with bimetallic regulator at upper right and tube tray in the foreground.

in scientific work. We, therefore, submit the description of a few that we have found practical and valuable to our work here, in the hope that others may profit thereby.

The first item is a water-bath for blood agar plating. It consists of a copper tank 21 inches long, 10 inches wide by 7 inches high at the "eaves" placed on galvanized iron legs 4 inches high and having a cross brace of galvanized iron under the bottom to give it support. There is a removable ⅓ pitch peak roof having a rod down the "comb" to act as a hinge and a hole cut in one end to accommodate a bi-metallic gas thermo regular. There is a double-decked tray that fits inside that will accommodate 72 tubes (6 tubes wide by 12 tubes long). The upper hole is 1 inch in diameter and

*From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Ill.
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Fig. 2.—Glassware dryer with doors open and showing trays of glassware.

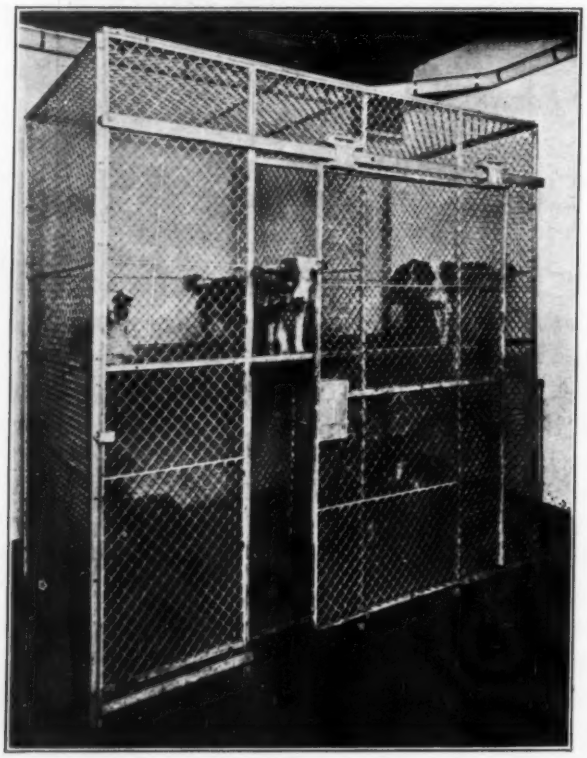

Fig. 3.—Showing dog cages in use.

½ inch apart, while the bottom is ⅜ inch in diameter. The heat is supplied by a micro-burner.

This apparatus affords great satisfaction in making blood agar plates, because the temperature can be adjusted to 45° C. and the blood and agar left together indefinitely before plating.

Another item that we deem worthy of mention is a live-steam dryer. It consists of a large galvanized iron box 4 feet wide by 3 feet deep by 5 feet high and has 4 shelves made of strap iron one foot apart. The heat is supplied by a coil of live steam pipes placed at the bottom. After washing the wet glassware is put into baskets 6 inches high by 12 inches wide by 24 inches long which are placed on the shelves within the drier. This affords a satisfactory means of preparing glassware for use.

The last item* we find to be a convenient means for the housing of dogs. It consists of a meshed enclosure 4 feet wide by 8 feet long by 8 feet high, and is divided by a partition into two halves each of which has two or three galvanized iron bottoms depending on whether two or three compartments each are desired. The metal trays of 14 gauge galvanized iron are grooved and sloped to the center of the partition where a funnel is located draining into a drain pipe that empties into a pan or floor drain. There is a sliding door on an overhead rail fastened by a latch and made to slide either way, permitting access to either side. A temporary door bearing four hooks may be used to cover the cell that is not being used.

The meshing is composed of ⅛ inch hot galvanized iron lock-mesh wire and is supported by strong angle irons at the corners and points of stress. This particular type of cage is the more desirable because it is almost completely made up of "stock" articles used in making wire fences and bank cages.

A METHOD OF PREPARING UNIFORM STAINS†

By C. Creston Collins,‡ Toledo, Ohio

NOT having observed in literature any mention of such a practice as I have found to be absolutely necessary in obtaining uniformly acting stains, more particularly blood stains, I am herewith giving a notation of my findings.

I will consider primarily the technic in making Wright's stain for blood preparations: Of course the usual precautions in obtaining a good dye and a chemically pure absolute methyl alcohol (free from acetone) must be observed. Even then laboratory workers and supply houses do not seem to

*The last item has been constructed for us by the Page Steel and Wire Company, 208 South LaSalle Street, Chicago, Ill., while the first two items may be made by any tinsmith or metal worker.

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‡Bacteriologist, Flower Hospital.

be able to produce stains of unvarying accuracy. The method of overcoming this difficulty, however, is very simple and quite definite. *The mixture should be placed in an amber bottle, stoppered with a greased glass stopper, shaken for twenty minutes each day for ten consecutive days, and allowed to stand for five days more before using.* The continual agitation, of course, merely serves to break up the molecule so that the maximum strength of the dye is obtained. This is a minimum time for the obtaining of uniformly perfect results.

Using this technic and preparing the stain in the proportion recommended by Wright, a stain can be produced the staining time of which will be: stain for 15 seconds and dilute with distilled water (double distilled free from ammonia is even better) for 45 seconds.

A large quantity can be prepared at one time, and it will keep perfectly in such a bottle as mentioned above for months (and even years).

Although personally I use this method in preparing all stains, it will be found to be of particular value in the preparation of Wright's, Jenner's, et al., and saturated solutions of dyes.

THE USE OF BUFFER SOLUTIONS IN STAINING BLOOD FILMS WITH WRIGHT'S STAIN*

By Russell L. Haden, M.D., Kansas City, Kas.

PROBABLY every laboratory worker has had difficulty at times in staining blood films satisfactorily with Wright's stain by the usual technic. Often the red cells take on a "muddy" blue color and the white corpuscles lack sharp differentiation. Sometimes even the plasma will take up the blue stain. Such preparations are exceedingly unsatisfactory for accurate qualitative examination and for differential leucocyte counts.

In the preparation of Wright's stain the active basic constituent, methylene azure, is formed by heating methylene blue in an alkaline solution. In staining blood films, the "muddy" blue preparations are due to an excess of alkali. One method of neutralizing the undesirable effect of the alkali is to add a small amount of acetic acid to the distilled water with which the stain is diluted. It is not an easy matter, however, to add just the optimum amount.

A simpler method of obtaining the correct reaction of the mixture of water and stain is by the use of a buffer solution instead of distilled water for diluting the stain. The buffer absorbs the excess alkali without changing the reaction of the staining solution. The use of buffer solutions for this purpose was first suggested by McJunkin in describing a new polychrome benzidine stain for blood.[1] It seems, however, that few laboratories

*From the Department of Medicine, University of Kansas School of Medicine, Kansas City, Kansas.
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have made use of them. The buffer solution must be slightly acid. McJunkin employs a solution with a hydrogen-ion concentration of 6.4. We have found this quite satisfactory, although solutions with the hydrogen-ion concentration varying from 6.0 to 6.6 may be used.

The simplest buffer solution with a hydrogen-ion concentration of 6.4 is one made from acid monopotassium phosphate and dibasic sodium phosphate. The potassium phosphate is easily procured in pure form. The dibasic sodium phosphate crystallizes with 12 molecules of water of crystallization ($NA_2HPO_4 . 12H_2O$). On exposure to air it loses 10 molecules of water and forms $Na_2HPO_4.2H_2O$. Since the water content of these two salts is somewhat uncertain, it is best to use anhydrous dibasic sodium phosphate (Na_2HPO_4) which is sold as "Sorensen's salt." If this is not available the crystallized dibasic sodium phosphate may be exposed to the air for two weeks, during which time it should be completely converted into $Na_2HPO_4.2H_2O$. The phosphate buffer solution with hydrogen-ion concentration of 6.4 is made by dissolving 6.63 grams of chemically pure recrystallized acid potassium phosphate and 2.56 grams anhydrous dibasic sodium phosphate, or 3.20 grams $NA_2HPO_4.2H_2O$ in one liter of distilled water. If M/15 solutions of the two salts are available the desired solution may be made by adding 73 cc.. of the M/15 acid potassium phosphate (9.078 grams per liter) solution to 27 c.c. of the M/15 dibasic sodium phosphate (9.47 grams, Na_2HPO_4, or 11.876 grams, $Na_2HPO_4.2H_2$, per liter) solution.

The following technic is employed in staining the blood film. The Wright's stain is run on and left for 1 minute. An equal number of drops of the buffer solution are added and allowed to remain for four minutes. The film is then washed with distilled water, dried, and examined.

The red cells should be a clear buff color. The granules and nuclei of the white cells are brought out brilliantly. The platelets are a deep purple. Occasionally specimens of Wright's stain are obtained which stain films satisfactorily by diluting with distilled water. With such a preparation it is, of course, unnecessary to use the buffer solution.

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DETERMINATION OF UREA NITROGEN IN BLOOD WITHOUT AERATION*

By Henry M. Feinblatt, M.D., Brooklyn, N. Y.

THE elimination of aeration from the technic of the urease colorimetric method of urea nitrogen determination in blood would materially simplify the procedure. Accordingly, comparative estimations were made with and without aeration. In collaboration with Dr. E. A. Stoller of the Long Island College Hospital, the writer performed 75 parallel tests, and the differences were no greater than could be accounted for by individual variations in colorimetric readings. The author therefore believes that aeration may safely be omitted from the urease method.

The writer employs the following method: The blood filtrate is prepared in accordance with the technic of Folin and Wu.† To 5 c.c. of the filtrate in a test tube 25 mm. in diameter, add 1 c.c. of urease solution or a piece of filter paper impregnated with strong urease. Add 2 or 3 drops of pyrophosphate solution (buffer mixture), and immerse the tube in warm water at 40 to 55 degrees C. for 5 minutes. After incubation transfer the contents of the tube to a tube marked at 22.5 c.c. and at 25 c.c. Rinse the first tube with distilled water and add the rinsings to the second tube. Dilute with distilled water up to the 22.5 c.c. mark. Add Nessler's solution up to the 25 c.c. mark, and read against the standard.

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†Folin, O.: Laboratory Manual of Biological Chemistry, 1922, p. 229.

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EDITORIALS

The Journal of Laboratory and Clinical Medicine

THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE was founded in 1915, to fill a need at that time quite obvious both to clinicians and research students. Then as now, there was an abundance of medical periodical literature composed of regional society organs and of more highly specialized journals devoted to the various subdivisions of medicine. The latter dealt with the laboratory sciences, as bacteriology, pathology, biochemistry, or with purely clinical work. There was no nationally recognized periodical primarily correlating practical laboratory developments and observational clinical experience.

The editor-in-chief and the publisher, recognizing the desirability of some such medium for discussion, established THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE. During the ensuing eight years the original purpose has been constantly in mind and in the steady development of the three departments of the Journal, the balance of growth has been maintained so that no one branch has progressed at the expense of others.

The division devoted to original articles has become a forum for the presentation of work of purely scientific interest, as well as that of pre-

dominantly clinical import. Nevertheless the editorial policy of accepting original work of interest equally to the clinician and the laboratory worker, has made this department one of great value to medical men in general. Readers of the Journal require no illustration to substantiate this statement, but a simple example might be mentioned with regard to insulin, the discovery of which is of unusual interest "on both sides of the house." The first communication on this subject was made by Banting and Best in the pages of this Journal.

In the division of laboratory methods there have appeared new procedures of examination and study, improvements and simplifications of former methods, and communications in which such methods have been applied in the study of varied laboratory problems. This department has become particularly useful to the staffs of clinical laboratories, in hospitals and in private practice, for it is here that much of the new laboratory work makes its appearance. It provides opportunity for the description of new laboratory technic and distributes it to a laboratory audience.

The editorials have directed attention to recent developments of importance in all fields of medicine, and have been sufficiently comprehensive to allow a reasonably thorough grasp of the subjects discussed. As a rule, the editorial policy has been to discuss new work of value without passing final judgment upon its merits. Few of us nowadays find sufficient time to read any great volume of medical literature, particularly that outside the sphere of our special interests, and for this reason the scope of the editorial work has been made broad enough to include subjects of general interest in all fields. In so far as possible, through its editorial pages, the Journal has served its readers as a general medical journal in which work of questionable value has been eliminated, and the opinions and conclusions of diverse observers have been correlated.

Under the guidance of Dr. Victor C. Vaughan and with the able cooperation of his associated editors, we may quite safely say, THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE has developed into the standard journal in this country for the publication of new work and new ideas on laboratory methods, and for the exposition of those subjects of interest both to the clinician and to the laboratory worker.

The editorial policies which have placed this Journal in its present position among medical periodicals will be continued in the future. The new editor bespeaks the continued cooperation of the editorial associates, and the support of the many contributors who have filled these pages. Articles from new contributors coming within the scope of the Journal will be gladly received and given due editorial consideration.

—*W. T. V.*

Fusospirillary Symbiosis

THE close association of a fusiform bacillus and spirochete in Vincent's angina has raised the question whether we are dealing with a true symbiosis, or two phases in the life cycle of a single microorganism. The constant association of two distinct species of bacteria is somewhat at variance to prevailing bacteriologic concepts. Of course the microorganisms might exist independently and cause pathologic disturbance only when, by chance, they have opportunity to multiply together. That these germs which are always found in Vincent's angina are true pathogens, is indicated by the fact that (1) they are rarely found associated in the healthy mouth; (2) they are often seen in infection of the gums and tonsils; (3) that in diseases such as epidemic tonsillitis, all cases show the same organisms, and resemble each other clinically; (4) that neoarsphenamine causes their rapid disappearance; (5) that the lesions subside coincidently with the disappearance of the organisms; and (6) that the microorganisms, when injected into guinea pigs, uniformly produce lesions of a constant character similar to those observed in man. Several morphologic types of B. fusiformis are recognized, and many spirochetal organisms have been isolated from the buccal mucosa. Kritchevsky and Seguin hold that a symbiosis exists between two independent microorganisms, and that while the fusiform bacillus appears to be specific, the spirochetal element is not. Following guinea pig inoculation with any of three spirochetes, together with a pure culture of B. fusiformis, they obtained constant local reactions, frequently a generalized spirochetosis. The injection of spirochetes only, or of fusiform bacilli alone, caused no reaction.

Pure cultures of fusiform bacilli have been studied for spirillary forms. None was observed by Veillon and Zuber, Ellermann, or by Weaver and Tunnicliff. Tunnicliff later found that after cultures had stood for longer intervals, spirillary forms did appear. They were present in any considerable number for a few days only, and required special staining methods. These facts account for their having been previously overlooked. Larson, Barron and Klimenko have made similar observations. Some investigators have explained Tunnicliff's later findings on the assumption that the cultures were not pure. This possibility seems, however, to have been carefully eliminated. The author has recently studied a pure culture of B. fusiformis isolated from a normal tonsil. Her report is supplemented by unusually good photomicrographs, which quite clearly show spirillary forms occurring in this pure culture. The fusiform bacillus appeared quite pleomorphic, forming straight and wavy, nonmotile filaments. With Giemsa stain the filaments and individual bacilli stained blue, while certain incorporated bodies were bright pink in color. Under certain conditions the latter bodies appeared to fall out of the threads and later develop into motile spirilla. Sometimes the spirals, developing from the same pink bodies, remained in the filament or cell, to emerge later, either through the end of the thread or after its rupture. The spirilla ultimately degenerated, while elongated, slightly pointed blue bodies appeared within, resembling the short fusiform bacilli

seen in young cultures. A symplastic stage was also observed, when a mass of bodies was found from which bacilli and threads emerged.

Tunnicliff believes that the fusospirochete combination represents different forms in the life cycle of a single organism. If this interpretation be correct the specifity of Vincent's angina and kindred infections is more readily comprehended.

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—*W. T. V.*